Development and analysis of environmentally neutral, biodegradable, novel flocculants for drinking water treatment

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MSc

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Declaration

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Abstract

The objective of this work is a systematic study of the use of oleaginous-seed press-cake extracts of *Moringa oleifera lam*, hemp, sunflower, rapeseed, strawberry, lime and raspberry as bioflocculants for the removal of suspended solids in the process of coagulation and flocculation. A quantitative method for the analysis of the coagulation and flocculation process was developed to test the activity of the proteinaceous extracts, based on a spectrophotometric assay using kaolin to create the turbid water samples. This method could be used to measure both the initial flocculation activity rate as well as the total level of turbidity removal. Extracts of the press-cakes were tested for flocculation activity over a range of physical and chemical conditions, including initial turbidity, coagulant dosage, pH, ionic strength, and reducing agents dithiothreitol (DTT) and proteases (Proteinase K). Of the extracts studied, all showed significant levels of coagulation and flocculation activity with the exception of strawberry, raspberry and lime extracts. The level of turbidity reduction was calculated and in the order of decreasing activity, the most effective bioflocculants were found to be Moringa, sunflower, hemp and rapeseed. These results indicate that many common oleaginous-seed press-cake extracts show potential as bioflocculants in water treatment processes.

The first section of this work used a simulated Jar test assay, which required the samples to be taken off-line and measured in a spectrophotometer. This process made accurate measurement of sedimentation often difficult and was extremely time consuming.
This has led to the much needed continuous-reading on-line turbidity meters to be attached to the standard jar test apparatus. Therefore, the main aim of this section of the project was to develop and validate a new on-line quantitative process for the analysis of flocculation activity by attaching six Mettler Toledo Turbidity Transmitter TRB 8300 probes to a data acquisition system (Lab View). Each of the probes was then placed inside the vessels in the Phipps and Bird PB-700 Jar Test apparatus. Instrument calibration and process validation were examined by setting up a study on a multipoint calibration, limit of detection, and sensitivity. The effect of interfering light, flocculant colouring effects on the readings from the probes, measurement of flocculation rates and change in turbidity was examined. The results have demonstrated that all probes work within a 95% confidence interval for the measurement of the flocculation assay. This result will aid the efficiency and accuracy of the experimentation by allowing six independent on-line measurements of flocculation and sedimentation activity to be determined in parallel rather than the original single Jar test assay.

Abbreviations

BF's	Biological flocculants
pI	Isoelectric point
DIT	Dithiothreitol
RPM	Row's per minute
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
EDTA	Ethylenediaminetetraacetic acid
OD	Optical density
BOD	Biological oxygen demand
PPM	Parts per million
FR	Flocculation rate
NTU	Nephelopmetric turbidity units
LED	Light emitting diode
LOD	Light of detection
ANOVA	Analysis of variance
SPSS	Statistical Package in Social Science

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Chapter 1

1. Introduction

1.1 Coagulation and Flocculation

1.1.1 Coagulation

"The term coagulation describes the effect produced when certain chemicals are added to raw water containing slowly settling or nonsettleable particles. The chemicals hydrolyse and neutralise the electrical charges on the colloidal particles, which form agglomerations termed flocs, which will be removed by clarification and filtration" (Environmental Protection Agency Ireland, 2002). Suspended solids can be of mineral origin, such as sand, silt and clays, or of organic matter such as dead and decaying plants and animals. Colloidal particles are also suspended solids; however they are of much smaller in size and settle at a much slower rate as they find it very difficult to settle naturally. Coagulation occurs in the final stage of the solids-liquid separation: settling, floatation or filtration process (Degremont, 1991). For effective coagulation there must be attractive forces between particles and filter media so that attachment can occur. Colloidal particles can interact with each other and can either attract to each other or more commonly they repel each other. If the colloidal repel each other they are said to be colloidally stable in so far as they have the ability to remain dispersed over long periods of time (Mara and Horan, 2003).

There are two very important types of interaction between particles in water: the first being the van der Waals attraction, which relates to the structure and form of colloids as well as the type of medium (Degremont, 1991). Between equal spherical particles of radius a, separated by a distance d, the van der Waals interaction energy V_A, is given approximately by:

$$V_A = -\frac{Aa}{12d}$$

Where A is the Hamaker constant, which depends on the properties of the particles and water. For water suspension, this ranges between about 5 and 100 x 10^{-21} J (Mara and Horan, 2003). The lower values are typical of biological particles such as bacteria and

algae whereas dense mineral particles have much higher values (Mara and Horan, 2003). The second important interaction is the electric repulsive force which relates to the surface charge of the colloids (E_B). The stability of a colloidal suspension depends on the balance between the forces of attraction and repulsion. The energy level of which is:

$$E = E_A + E_B$$

Where E_A is van der Walls energy and E_B electrostatic energy (Degremont, 1991). As the colloids in wastewater mainly have a negative charge it is necessary to destabilize the suspension in order to overcome the energy barrier E_s. To achieve this, hence promote agglomeration of the colloids it is important to destabilise the colloids by reducing the electrostatic repulsive forces (Henze e*t al*., 2000). The negatively charged colloids attract a cloud of positively charged ions around it, thereby neutralizing the surface layer, an electric double layer is produced (Henze e*t al*., 2000; Sundstorm *et al*., 1979). The zeta potential determines the moving of colloids and their electrical potential between these two layers.

In order to promote coagulation it is usually necessary to reduce electrical repulsion between particles. Increasing ionic strength by the use of adding salts to the water causes a reduced repulsion between the particles and possibly some reduction in zeta potential. This is known as indifferent electrolytes (Sundstorm *at el*., 1979). Therefore it is this addition of the salts that optimise coagulation. If the water treated was of constant quality and character as groundwater from a deep well, it would be possible to calculate from analyses of the water the optimum chemical conditions and dose of coagulant required. However, surface water is never as constant in quality and character as the temperature is constantly changing, pH levels fluctuate and even particulate levels can change with wind strengths (Ndabigengesere *et al*., 1995). With all these different factors, it is essential to take them into consideration in choosing what coagulants to use and the different concentrations of coagulants. To determine the optimum chemical conditions in terms of coagulation dose and pH for treatment of the water concerned are assessed by means of the jar test.

1.1.2 Flocculation

"Flocculation is a process of gentle water movement that promotes the gathering together of the small floc particles (microflocs) produced by coagulation into larger masses better suited for removal by clarification process" (Environmental Protection Agency Ireland, 2002). Flocculation is the main purpose of carrying out coagulation, without coagulation flocculation would not exist and solid-liquid extraction could not happen. The flocculation process provides conditions for contact between particles to form flocs for ease of removal in water treatment. Floc formation is controlled by the rate at which collisions occur between particles. The purpose of flocculation is to create a floc to a suitable size and density so it can be removed by sedimentation and filtration (Mayer and Wells, 2011). The best floc sizes ranges from 0.1mm to about 3mm, depending on the type of removal process used. The smaller the floc is the more suited it is to removal by filtration and the larger the floc is the more suited it is to clarification. Some flocculation can be accomplished by the turbulence resulting in the roughness in the conduits or channels (Environmental Protection Agency Ireland, 2002).

For successful flocculation the introduction of another agent is often needed to promote the formation of the floc, called a flocculant or flocculant aid. Inorganic polymers and natural polymers were the first flocculants to be used; however the appearance of a wide variety of synthetic polymers has changed flocculation drastically. These synthetic polymers consist of long-chain macromolecules obtained by the association of synthetic monomers, which pass electrical charges or have ionisable groups (Thomas *et al*., 1999). These are products that have a very high molecular weight (10^6-10^7), enabling them to attain remarkable performance levels that are usually higher than those attained with natural polymers (Degremont, 1991). The flocculant addition does not take place until the coagulant stage is over; the time required for coagulation depends on the type of the colloid as well as the temperature and pH of the water. The reason synthetic flocculants are used is that as well as being more effective than natural flocculants, when combined with modern separation techniques they can result in the production of a very dense sludge that can be directly treated in the dewatering stage.

Flocculation efficiency may be expressed as follows:

$$e = k\, C^{\alpha}\, G^{\beta}\, t^{\gamma}$$

Where, e is the flocculation efficiency (this factor relates to the floc formed of to the quality of the settled water), C is the sludge concentration in flocculation reactor, G is the velocity gradient, t is the contact time and $\alpha, \beta, \gamma > 0$. With the increase in C (contact mass) this increases that the probability of collisions will take place inside the reactor resulting in a more efficient flocculation (Degremont, 1991). Solids contact units give a great number of advantages such as, enhanced flocculation, completion of specific reactions, and higher organic matter removal by absorption on the floc and savings on chemical reagents (Environmental Protection Agency, Ireland, 2002).

2 Chemicals used in coagulation and flocculation

Chemicals used in coagulation and flocculation are referred to as either primary coagulants or as coagulant aids. The coagulant aids can be used to condition the water to add density to slow-settling flocs or toughness so the floc will break up for the use of the primary coagulants. Primary coagulants cause the particles to become destabilised and begin to clump together. The reagents currently used in coagulation and flocculation are inorganic products, natural polymers and synthetic polymers. The most widely used coagulants are aluminium or iron salts and less frequently used coagulants used are cationic polyelectrolytes. Aluminium and iron salts (Pritchard *et* al., 2010) are used because they are effective, relatively low cost, widely available and easy to handle, store and apply. Aluminium sulphate is still used widely even though there has been much concern over the possible effects to human health, such as Alzheimer's and possible have strong carcinogenic properties (Crapper *et al*., 1976; Plat *et al*., 2007).

2.1 Aluminium Salts

When aluminium sulphate is added to water, the aluminium ions enter into a series of complicated reactions. The aluminium ions become hydrated, meaning that water molecules attach themselves to the aluminium ions. In addition, anions present in the water, such as hydroxide and sulphate ions can attach to the aluminium ions. These reactions result in large, positively charged complexes having aluminium ions at their centre. These particles may have charges as high as +4. Following these reactions, a second type of reaction occurs, called olation, where metal ions form polymeric oxides in aqueous solution. This reaction involves the bridging of two or more of these large molecules to form even larger, positively charged ions. A typical molecule can contain eight aluminium ions, twenty hydroxide ions, and will have a +4 charge. Iron salts behave in a similar manner when added to water (Cape Canaveral, 1996).

The basic reaction when aluminium (Al^{3+}) is added to water is the formation of a precipitate of aluminium hydroxide with the release of some acidity

$$Al^{3+} + 3H_2O \leftrightarrow Al(OH)_3 + 3H^+$$

These hydrolysis reactions are strongly influenced by pH i.e. the higher the pH the greater the tendency for the formation of hydroxylated species. The uncharged hydroxide $Al(OH)_3$ has a very low solubility in water and hence forms a precipitate optimally around a neutral pH. A similar reaction occurs when ferric salts are added to water, although they are less soluble than $Al(OH)_3$ but precipitates over a greater range of pH (Mara and Horan, 2003). Aluminium sulphate (liquid or solid) is added in solid form as blocks, as granulated or kibbled alum and also in liquid form. The chemical formula of pure aluminium sulphate is Al_2SO_4 . $3{\cdot}18$ H_2O.

2.1 Iron Salts

Iron salts can also be used for coagulation; they work on much of the same principals as

aluminium.

$$Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+$$

There are two main types of iron salts that are commonly used ferric sulphate and ferric chloride. Ferric sulphate is supplied as a red-brown powder or as granules. Its chemical formula is $Fe_2(SO_4)_3 \cdot 9H_2O$. It is mildly hygroscopic but is difficult to dissolve and the solution is corrosive to aluminium, concrete and most steels. In a manner similar to the reaction with aluminium sulphate, the formation of a ferric hydroxide floc is the result of the reaction between the acidic coagulant and the natural alkalinity of the water, which usually consists of calcium bicarbonate, as expressed by the equation below, with the insoluble products (precipitates).

$$Fe_2(SO_4)_3 + 3Ca(HCO_3)_2 \leftrightarrow 2Fe(OH)_3 + 3CaSO_4 + 6CO_2$$

Timing of the addition of conditioning chemicals and coagulants, as well as of coagulant aids, has been found to be of great importance and is usually critical to effective clarification performance, satisfactory filter performance, as a consequence, and hence the quality of the final water. Suitable separation of the dosing points for the different chemicals and provision of suitable delay times between chemical additions can be of considerable importance in achieving optimum coagulation. A study by the Water Research Centre of the clarification of five different water types by flotation showed that dosing the coagulant chemicals directly into the raw water feed pipe gave improved quality in the flotation treated water, compared to dosing them into the flash mixer. The order of chemical addition had little influence on the treated water quality – either equal or slightly better results were obtained dosing the pH adjustment chemical first (Environmental Protection Agency Ireland, 2002).

3 Jar Tests

Jar test may be used to study the effects of dose of coagulant chemicals and pH on settled water quality. The amount or dosage of a precipitant, coagulant and/or flocculant required to precipitate and remove metals in wastewater solutions is not only dependent

on the concentration of such chemical in solution, but also on several other factors. To optimise the dosage (Water Specialist Technologies, 2005), the following parameters must be considered:

- The solution pH.
- The chemical used to adjust the pH.
- The different types (and concentrations) of metals present in solution
- The coagulants and flocculants used.
- The ionic strength of the solution.

Jar testing is a method of simulating a full-scale water treatment process, providing system operators a reasonable idea of the way a treatment chemical will behave and operate with a particular type of raw water. Because it mimics full-scale operation, system operators can use jar testing to help determine which treatment chemical will work best and the optimum dosage of chemical required. The sample is then firstly stirred at a high RPM for two minutes to mix the flocculant/coagulant with the suspended particles. It is then left to mix at a low RPM for five minutes so that the promotion of formation and development of flocs can occur and finally the mixing is ceased in order to allow the settlement of flocs.

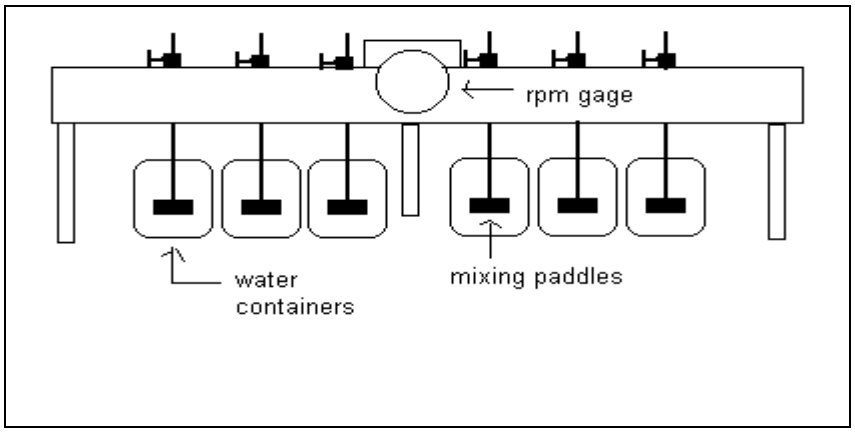

Figure 1. Apparatus for Conducting Jar Test Assays (Olom, 2009)

4 Possible Biological Alternatives

Biological flocculants (BFs) for turbidity removal have been investigated worldwide in recent years. The results demonstrated that BFs were able to eliminate turbidity from kaolin clay solution over a wide dosage range. The removal efficiency with BFs reached 86 % on average, lower than 95 % with $Al_2(SO_4)_3$ and 96 % with $Fe_2(SO_4)_3$, respectively. For bioflocculants, bridging flocculation other than charge neutralization should be responsible for turbidity removal. The combined applications of BFs with $Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$ increased the over-all turbidity removal up to 97 %. It was also shown that combination of BFs and $Fe_4(SO4)_3$ was effective for removing turbidity from raw water. This study provides a proof-in-concept demonstration of BF's for water purification, which can in part reduce operational costs in coagulation treatment, as well, effectively reduce the concentration of residual metallic elements (e.g. aluminium) in coagulated solution (Ma *et al*., 2008). To date some research has been done on natural flocculants such as chestnut and acorn (Šćiban *et al*., 2009), common bean *(Phaseolus vulgaris)* (Antov *et al*., 2010), cactus (Zhang *et al*., 2006), *Cactus latifaria* and *Prosopis juliflora* (Diaz *et al*., 1999) however to date literature suggests that *Moringa oleifera lam* seeds and extracts have been the most successful natural coagulant. (Ghebremichael *et al*., 2005; Jahn, 1988; Ndabigengesere *et al*., 1995)

Moringa oleifera lam can be found in many tropical countries around the world and are used now in countries such as Sudan and Johannesburg as a potent coagulant (Jahn, 1979). It is of special interest in traditional water-cleaning methods in Sudan where the seeds of Moringaceae are preferred. Moringaceae, although very similar to the Capparidaceae, from a family of their own including one genus (Moringa) and 14 species (Okuda *et al*., 2001) of which of which *Moringa oleifera lam* has gained considerable importance as flocculant and is known as a plant containing an active coagulating compound (Jahn, 1988). To optimise the use of these cheap native flocculants in developing countries, great efforts have been made worldwide to research into these seeds (Jahn, 1988).

Earlier studies recommend the use of Moringa seed extracts as coagulant for water treatment in African and South Asian countries where the plant is considered indigenous (Jahn, 1988; Schulz *et* al., 1983). If *Moringa oleifera lam* is proven to be active coagulant, and to be safe and inexpensive, it is possible to use it widely for drinking water and waste water treatment in other countries as well. *Moringa oleifera lam* may

become one of the cash products bringing more economic benefits for the producing countries. The seeds of *Moringa oleifera lam* and *Moringa stenopetala* tree have been described as comparable to alum in coagulating activity, being used in Sudan for household water purification (Jahn, 1991; Ndabigengesere, *et al*., 1998).

There have been many studies on Moringa seeds in which its proteinaceous flocculant material was identified. In first investigations Barth *et al*. identified proteinaceous fractions as the active principle present in the seeds (Barth *et* al., 1982). Gassenschmidt *et al*. were able to isolate a peptide with coagulating activity from seed material and to determine its primary sequence (Gassenschmidt *et* al., 1995). They found that the seeds contained a cationic peptide composed of 60 amino acid residues with a molecular weight of 6.7 kDa that they termed Mo 2.1 (Gassenschmidt *et al*., 1995). There has been some conflicting data reported for the molecular weight of these proteins, it has been reported that the molecular weight varies between 3 and 16 kDa. The seeds of M. oleifera also contain oil (22-40% w/w), proteins (36-38% w/w), glucosinolate (20-26% w/w) and, in significantly lower amounts, phytic acid and phenolic compounds (2-3% w/w) (Makkar *et al*., 1997). With these variations in results, the molecular mass and constitution of the active component present in *Moringa oleifera lam* seeds is still subject to uncertainties, as the separation of the seed components is difficult to achieve.

A major effort has been undertaken by many researchers to identify the active substances present in the seed extracts and to maximize the extraction of proteins, while glucosinolate and isothiocyanate received little attention. It has been shown that the coagulating activity has been attributed to a non-proteinaceous organic substance of unknown composition by Okuda et al. (Okuda et al. 2001). Okuda also reported that the extraction of an active compound can be improved by increasing the ionic strength of the solvent used (Okuda et al. 1999). However, results from Ghebremichael showed that extraction using salt solutions indeed increases the resulting concentration of total protein (Ghebremichael *et al*., 2005). This is not in accordance with the findings of Okuda et al. (Okuda et al. 2001), who suggest that the active component is of non-proteinaceous origin.

It has been claimed by Ghebremichael, that in the protein composition of extracts changed in dependency of the extraction method (Ghebremichael et al. 2005).

Extraction of Moringa seed coagulant has traditionally been carried out directly during the coagulation process by bringing the crushed seeds in contact with turbid water (Jahn SAA, 1979). For this reason it is possible that investigators initially focused on extraction processes using tap water or distilled water (Barth *et al*., 1982; Donli *et al*., 2003; Kalogo *et al*., 2002; Ndabigengesere *et al*., 1998). However, solutions containing buffering salt, such as NH_4HCO_3, phosphate or borate (Gassenschmidt *et al*., 1995; Tauscher, 1994) were used in order to keep pH values of the resulting extracts constant over time. Nevertheless, only the use of high salt concentrations (1M NaCl, KCl, $NaNO_3$ or KNO_3) resulted in significant differences in the concentrations of the coagulating substance (Okuda *et al*., 2001).

With the comparison of results from different researchers it has proven difficult to confirm the coagulation activities of the plant extracts; this may be due to the variability, inherent to natural materials collected in the wild. For example, coagulating activity is related to the initial ratio of seed material to extraction solution (Muyibi *et al*., 2002; Ndabigengesere *et al*., 1995), consequently making it difficult to judge the extraction efficiency. In addition, the protocols for the determination of coagulation activity differ from one author to another making it difficult to compare different extracts. It has been found in this research that coagulation/flocculation is extremely difficult to quantify, as many factors play a role (Gregory, 1989), including salt concentration, pH, etc. on the solvent, as well as the charge and pK_a values of functional groups present on the particle surface. It is clear, that factors in the sample solution can potentially affect the outcome of the experiments and control experiments have to be undertaken. Therefore, the set-up of a coagulation experiment should aim at the use of standardised materials and conditions.

This project aims to illustrate along with the existing knowledge of the potential of using protein extracts from Moringa but also the potential of using protein extracts from Sunflower and other presscake extracts as a potent bioflocculant.. The first section of this work used a simulated Jar test assay, which meant the samples needed to be taken off line and measured in spectrophotometer. Accurate measurement of sedimentation

can often be difficult as samples need to be taken off line and inserted to into a spectrophotometer which can be extremely time consuming.

This has led to the much needed continuous reading online turbidity meters to be attached to the standard jar test apparatus. Therefore the main aim of the second section of the project was to develop and validate a new on-line quantitative process for the analysis of flocculation activity by attaching six Mettler Toledo Turbidity Transmitter TRB 8300 probes to a Data Acquisition (labview), each of the probes were then be placed inside the vessels in the pipps and bird PB-700 Jar Test apparatus. This will allow six an online measurement of flocculation and sedimentation activity to be measured at once rather than the original single Jar test assay.

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Chapter 2

A Study of the efficacy of novel bioflocculants, from oleaginous seed press-cake, on coagulation and flocculation and an investigation into the parameters affecting their activity

2.1. Introduction

Coagulation and flocculation are key steps in water treatment as these are the most effective was of removing turbidity from water. This involves the removal of suspended microparticulate matter by the addition of highly charged cations such as aluminium and iron salts, usually in the form of aluminium sulphate ($Al_2(SO_4)_3$) and ferric sulphate ($Fe_2(SO_4)_3$), commonly termed alum and ferric sulphate respectively, to water (Rossini *et al*., 1999; Pritchard *et al*., 2010b). These ions neutralise the electrical charges on the suspended solids and colloids which in turn form flocs (Ma *et al*., 2008). The flocs grow in size and merge and due to the increased mass and density, cause sedimentation which can ease their removal by filtration or clarification (Degremont, 1991; Thomas *et al*., 1999). Alum is the most widely used coagulant in water treatment; however there are perceived health affects due to their neuro-toxicity properties (Broin *et al*., 2002; Crapper *et* al., 1976; Platt *et al*., 2007). In addition alum and ferric sulphate salts create large sludge volumes and affect the natural alkalinity of the water by depressing the pH (Ndabigengesere *et al*., 1998; Haarhoff and Cleasby 1988; Pritchard e*t al*., 2010a). This is further complicated by the need to add polymers, such as acrylamide derivatives, to increase the efficiency of flocculation and sedimentation (Haydar and Aziz, 2009; Aguilar *et al*., 2005). In turn, this has led to safety concerns on the environmental fate of acrylamide, resulting in legislation to dispose of acrylamide-containing sludge by incineration (Council Directive, 1986).

Therefore, there is a much needed drive to find alternative coagulants and flocculants that are not only non-hazardous and environmentally acceptable but also cost effective. To date some research has been done on natural flocculants such as chestnut and acorn (Šćiban *et al*., 2009), common bean *(Phaseolus vulgaris)* (Antov, Šćiban, Petrović 2010), cactus (Zhang *et al*., 2006),*Cactus latifaria* and *Prosopis juliflora* (Diaz *et al*.,

1999) however to date literature suggests that *Moringa oleifera lam* seeds and extracts have been the most successful natural coagulant (Ghebremichael *et al*., 2005; Jahn, 1988; Ndabigengesere *et al*., 1995). It has been reported that soluble dimeric cationic proteins from *Moringa oleifera lam* extracts (Gassenschmidt *et al*., 1995; Ndabigengesere *et al*., 1998) are the active component for coagulation and flocculation in drinking water treatment. These proteins are reported to be non-toxic and have a high isolelectric point (pI) of 10.5- 11.0 (Gassenschmidt *et al*., 1995; Ndabigengesere *et al*., 1995), making them effective biodegradable cationic polymers, which it has been suggested, may be used as natural coagulants and flocculants for the removal of turbidity in drinking water treatment, especially in developing countries, where the plant grows readily (Pritchard *et al*., 2010a). It has been reported that the extracts from *M. oleifera* remove between 80 and 99% (Muyibi and Evison, 1995; Ndabigengesere *et al*., 1995) of the turbidity, have negligible effect on the pH of the water and produce up to five times less sludge than alum (Ndabigengesere *et al*., 1995).

The use of *M. oleifera* as a bioflocculant has some major disadvantages such as, it is an oleaginous plant found only in sub-tropical countries where it is widely used to produce oil for domestic and cosmetic use (Ricardo, 2012). It does not grow well in more temperate climates, consequently importing would be prohibitive to European countries. The main aim of this work was to screen a range of oleaginous plant seed press-cakes from plants including, hemp, rapeseed, sunflower, strawberry, lime and raspberry which are commonly found in developed and developing countries, to see whether such extracts also exhibit flocculation activity. Press-cakes are the waste product after the oil has been extracted from the seed (Katayon *et al*., 2006) and as such are a much cheaper alternative to seeds. *M. oleifera* press-cake extracts are believed to contain cationic peptides with an unusually high pI which are responsible for the flocculation activity. Since the extraction procedure for the proteinaceous components from *M. oleifera* (Gassenschmidt *et al*., 1995) has been documented, a similar extraction procedure was employed for all of the press-cakes under investigation (Okuda *et al*., 1999).

The aim of this study was the quantitative analysis of extracts from *M. oleifera* and other oleaginous press-cakes for flocculation activity and it was essential to have a standardised, reproducible flocculation assay. This was developed by combining a jar

test apparatus with a UV-VIS spectrophotometer. Use of this method ensured that the results could be quantified in order to determine the optimum flocculation activity of the protein extracts from the various press-cakes against reference solutions of aluminium sulphate (8%, w/v) and ferric sulphate (11.7%, w/v).

Given that the environments to which coagulants and flocculants are exposed can greatly affect flocculation activity, a range of physico-chemical parameters including initial turbidity, coagulant dosage, pH, and ionic strengths were tested. As far as the author is aware protein extracts from press-cakes of sunflower, hemp and rapeseed have not been previously reported as exhibiting flocculation activity. In order to establish whether the active component from the extracts is indeed proteinaceous, a range of protein denaturing conditions were employed, including boiling the proteins and the addition of the reducing agents dithiothreitol (DTT) and proteinase K, to establish whether activity resided in the intact proteins.

2.2 Materials and Methods

2.2.1. Protein extraction method

M. oleifera, sunflower, strawberry, raspberry and lime press-cakes were purchased from Earthoil (UK). Rapeseed and hemp press-cakes were purchased from Harnett's Oil (Northern Ireland). Alum (aluminium sulphate, 8%, w/v) and ferric sulphate (11.7%, w/v) were purchased from Acorn Water Ltd., Ireland.

Press-cakes were ground to a fine powder using domestic blender and stored in the dark at room temperature. Press-cake powder (10 g) was added to 50 ml of mixed salt extraction solution containing 411 mM NaCl, 10 mM KCl, 7 mM $CaCl_2$ and 20 mM $MgCl_2$. All chemicals were of analytical grade and obtained from Fisher Scientific U.K.

The press-cake suspensions were incubated at room temperature with gentle agitation (50 rpm) on an orbital shaker for 1h. The suspensions were then centrifuged (Centrifuge

FL40R., Thermo Scientific, France) at 5,000 rpm for 15 min and decanted through a cotton cloth. The supernatant was transferred to a Duran bottle, sealed and incubated at 85 ºC for 5 min. The samples were allowed to cool to room temperature, centrifuged for 5 min at 5,000 rpm followed by sterile filtration (0.2 µm) and storage at room temperature in sterile darkened conditions. (Boucher, 2006)

2.2.2. Protein determination

Due to variations in the protein content of the press-cake extracts, the bicinchoninic acid (BCA) method (Pierce BCA protein assay kit, Thermo Scientific, USA) for protein determination was used to ensure that all flocculation activity could be expressed in terms of specific activity. BCA reagent (2 ml) was added to 0.1 ml of protein standard bovine serum albumin (BSA) and extracts and these mixed and incubated at room temperature for 2 h, before measuring OD using a spectrophotometer at 562 nm. A standard curve was prepared by plotting the absorbance at 562 nm against BSA concentration (0-2,000 µg/ml). Protein concentration in the extracts was determined by reference to the standard curve, and suitably diluted to ensure the protein concentration in the assay did not exceed 100 µg/0.1ml.

2.2.3. Synthetic Turbid Water

Synthetic turbid waters were prepared using a series of 3 buffers including 5 mM acetate, pH 5; sodium phosphate, pH 7 and Tris/HCl, pH 9. The ionic strength of the buffers was adjusted to 10 mM or 100 mM by the addition of NaCl. Kaolin (Fisher Scientific U.K.) was added to the buffer to the desired initial turbidity.

High particle concentrations increase the frequency of collisions thereby making flocculation more effective. In order to determine the optimum particle concentration for the assay and to determine the effect of particle concentration on activity, each buffer was prepared with 2 g/L, 1 g/L, 0.5 g/L, 0.250 g/L and 0.125 g/L of kaolin over three pH values and two ionic strengths. The kaolin suspensions were incubated for 24 h, at

room temperature, with gentle stirring to allow for complete hydration of the kaolin, prior to use.

2.2.4. Flocculation Assay

In order to quantify the flocculation activity a jar test procedure was developed using a 1 L beaker, a mechanical overhead stirrer and a spectrophotometer (Spectronic Helios Alpha UV-Vis, Thermo Fisher Scientific, USA)

One litre of buffer was placed in a beaker together with the required volume of flocculant, (protein extract, alum or ferric sulphate). The suspension was mixed at a high speed (300 rpm) for 2 min followed by a low mixing speed (50 rpm) for 5 min, and finally no mixing for 5 min. Samples (3 ml) were removed at intervals from the beakers throughout the experiment and placed in cuvettes inside a spectrophotometer and the optical density measured at 600 nm against a water blank. Since the particles flocculate and sediment with time, samples were removed from 2.5 cm below the surface of the suspension, in the centre of the beaker. This method was repeated for all seven presscakes over three pH (5, 7 and 9) and two ionic strengths (10 mM and 100 mM)

The sedimentation rate was calculated by determining the initial slope of the optical density from the point at which mixing was stopped (7 min after addition of flocculant). The total level of sedimentation was determined from the optical density of the buffer after addition of flocculant minus the optical density 12 min after addition of flocculant. The rate of flocculation was used to determine which flocculants formed flocs at the highest rate, whereas the sedimentation level was used to determine the density of the flocs and the ability to sediment within 12 min.

2.2.5. Determination of active component in extracts

In order to determine whether proteins within the press-cake extracts were responsible for the flocculation activity, 3 protein denaturing conditions were employed; addition of a strong reducing agent (dithiothreitol, DTT) to disrupt disulphide bonds, a protease enzyme (proteinase K) to partially digest proteins present and boiling to 100ºC.

2.2.5.1. Effect of DTT on flocculation activity

To 40 ppm of the protein extracts from *M. oleifera*, hemp, rapeseed and sunflower press-cakes, 0.1 M of DTT (Sigma Aldrich Ireland Ltd.) was added followed by incubation at 80 ºC for 20 min. The activity of the extracts was determined using the standard flocculation assay with 5 mM phosphate buffer, pH 7 and a kaolin concentration of 2 g/L.

2.2.5.2. Effect of proteinase K on flocculation activity

To 40 ppm of protein extract from *M. oleifera*, hemp, rapeseed and sunflower press-cakes were added 132 µl proteinase K (Sigma Aldrich Ireland Ltd.), 100 µl Tris/HCl, 150 mM NaCl, 2 mM $CaCl_2$ and 368 µl UPH_2O, followed by incubation at 37 ºC for 30 min. The samples were removed from the water bath and 50 mM ethylenediaminetetraacetic acid (EDTA) added to deactivate the enzyme. The activity of the extracts was determined using the standard flocculation assay in 5 mM phosphate buffer, pH 7 containing 2g/L kaolin.

2.2.5.6. Effect of boiling to 100 ºC on flocculation activity

Forty ppm solutions of the protein extracts from *M. oleifera*, hemp, rapeseed and sunflower were placed in a water bath at 100ºC for 20 minutes. The samples were removed from the water bath and allowed to cool to room temperature, followed by sterile filtration (0.2µm) and storage at room temperature in sterile darkened conditions until required.

2.3. Results and Discussion

2.3.1. Flocculation activity of alum and ferric sulphate

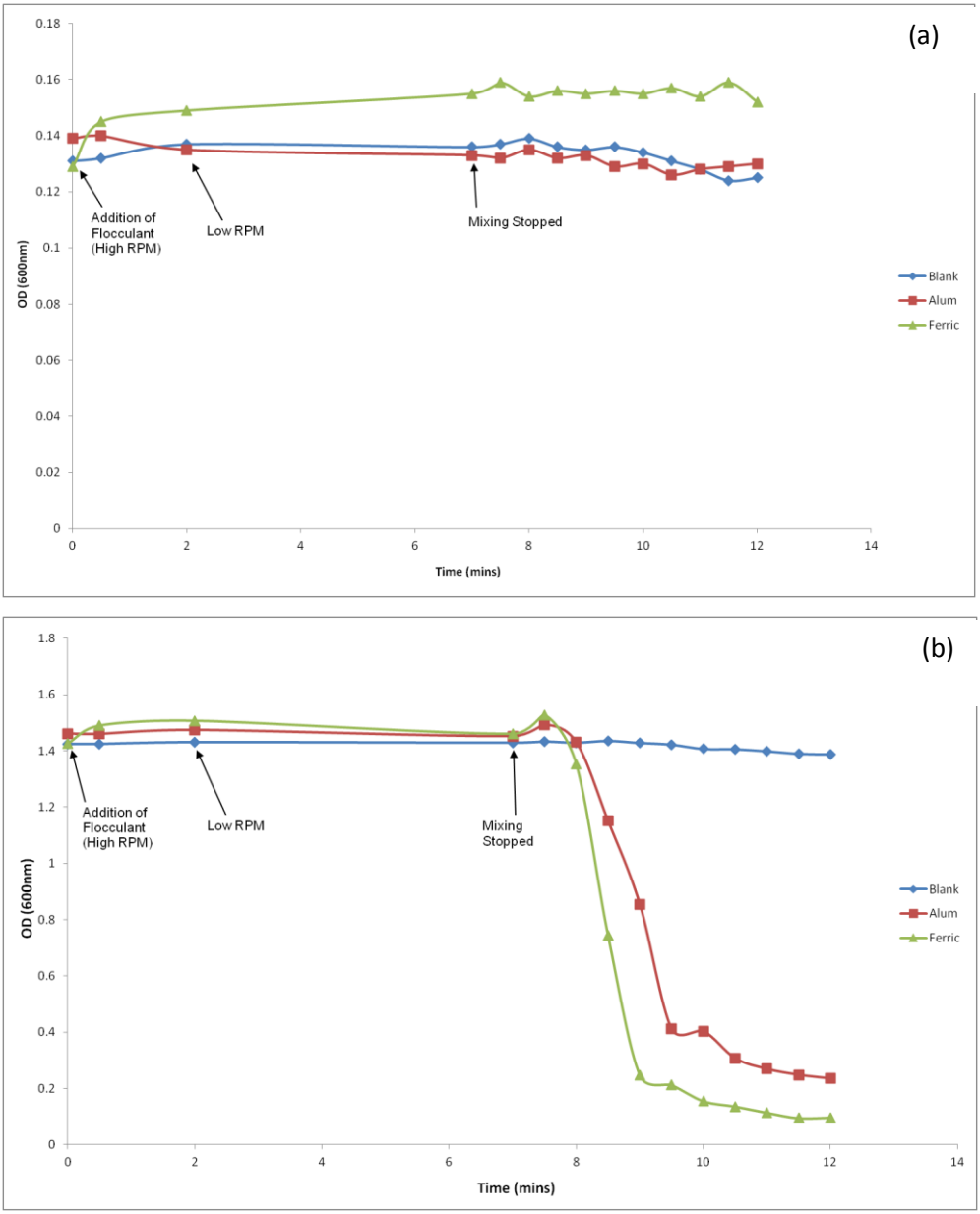


Figure 1. Flocculation activity by means of measuring the reduction in OD over a five min sedimentation time after mixing is ceased, for alum and ferric sulphate with (a) 0.125g/L kaolin and (b) 2 g/L kaolin.

Since alum and ferric sulphate are the standard chemicals used in water treatment facilities for coagulation and flocculation, the activity in the standard flocculation assay

was determined and used as a reference for the press-cake extracts. From Figures 1a and 1b, and Table 2 it can be seen that alum has a high flocculation rate and overall reduction in optical density (ΔOD) of approximately around 88 % at all kaolin concentrations above 0.250 g/L. There does, however, appear to be an optimum at 1g/L kaolin and only 20 ppm of alum, whereas the activity of ferric sulphate appears to be directly proportional to the kaolin concentration (Table 2). The results indicate that neutralisation of the charges occurs when the alum and ferric sulphate concentrations are of the order of 20 µg/g kaolin; below this level the size and density of the flocs do not enable rapid sedimentation.

2.3.2. Protein volume determined for coagulant dosage

The cationic proteins found in *M. oleifera* seeds and press-cakes have been documented to exhibit coagulation and flocculation activity (Ghebremichael and others 2005; Ndabigengesere, A., K.S. Narasiah, and B.G. Talbot 1995). In order to determine whether this is unique to *Moringa oleifera* or a property of proteins from other oleaginous plants, protein extracts from a range of oleaginous press-cakes were prepared using a standard salt extraction procedure (Okuda and others 2001a) and the extracts tested using a standard flocculation assay with kaolin suspensions.

Since the extracts may contain variable amounts of flocculant, the volume of extracts was varied in order to have between 0 and 80 ppm of protein in the assay (Table 1). The protein content was determined using the BCA assay with BSA as standard reference protein. The results (Table 1) show that, using the standard salt extraction method, *M.oleifera* extracts contained the highest protein content (68 g/L) followed by rapeseed (28 % less) and sunflower (56 % less) with the remaining extracts having 71-76 % less protein than that of *M. oleifera*.

Table 1. Protein concentration determined for the press-cake extracts together with the volume of the extracts (in ppm protein) used for the flocculation assays.

Presscake	**Protein (g/L)**	**20ppm**	**40ppm**	**60ppm**	**80ppm**
Raspberry	18.6	1080 µl	2160 µl	3240 µl	4320 µl
Strawberry	19.8	1010 µl	2020 µl	3030 µl	4040 µl
Hemp	19.0	1050 µl	2100 µl	3150 µl	4200 µl
Lime	16.1	1242 µl	2484 µl	3726 µl	4968 µl
Sunflower	30.1	664 µl	1328 µl	1992 µl	2656 µl
Rapeseed	49.0	408 µl	816 µl	1224 µl	1632 µl
Moringa oleifera	68.0	294 µl	588 µl	882 µl	1176 µl

2.3.3. Coagulant Dosage used in flocculation assays

The optimum dosage of coagulant for each environment was determined. Dosage is crucial in any water treatment plant, as over-dosing often leads to insufficient removal of turbidity as well as public health concerns with organic polymers and high operating cost whereas, under-dosing can also have a similar issue of insufficient removal of turbidity (Bouyer and *et al*., 2005). In order to compare the different extracts with alum and ferric, as well, as to define the optimum dosage of flocculant to be added, it was decided to standardise with respect to the protein content of the extracts. This is based on reports that it is the cationic proteins in extracts of *M.oleifera* which are responsible for the flocculation activity (Guibai and Gregory, 1991). The amounts of extract to be added to the standard assay to have a final concentration of 20-80ppm are shown in Table 1.

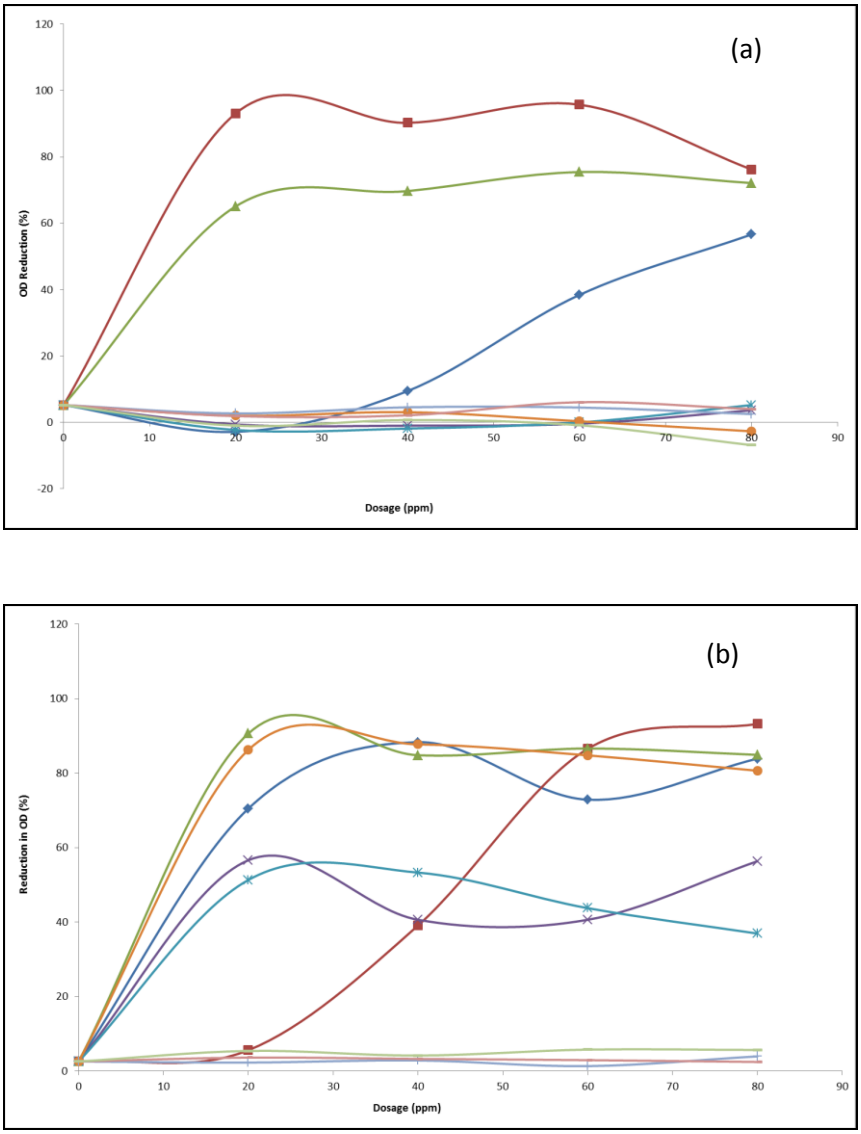


Figure 2. Shows flocculation activity of press-cake extracts dosages 0 ppm to 80ppm by measuring the % reduction on OD in (a) 0.5 g/L kaolin ; (b) 2 g/L kaolin.

Symbols: red filled squares, ferric; blue filled triangles, alum; green triangles, *M.oleifera* extract; purple crosses, hemp extracts; blue stars, rapeseed extract; orange circles, sunflower extract; grey crosses, strawberry extract; pink dashes, raspberry extract; green dashes, lime extract

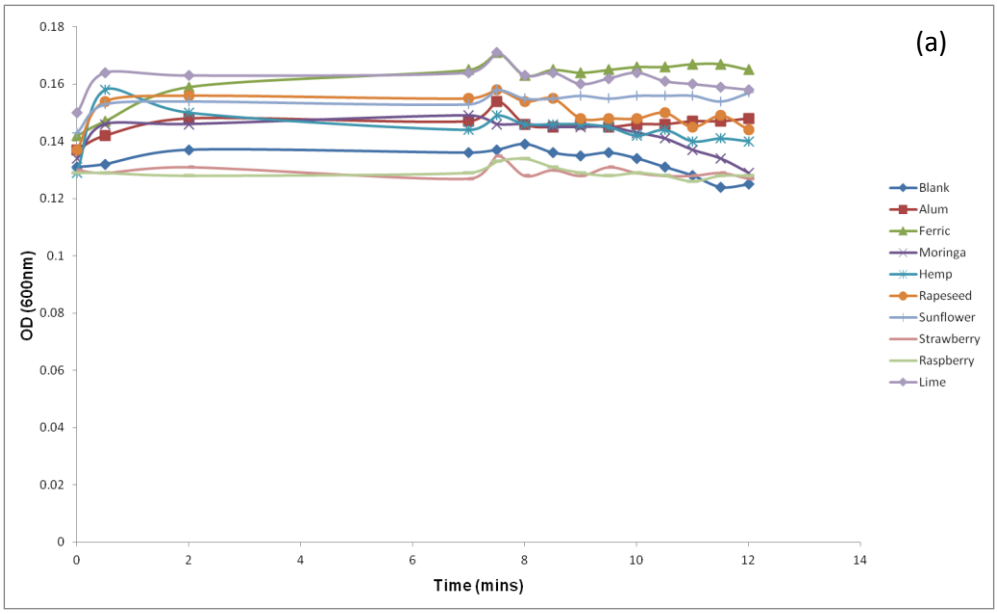
Table 2. Optimum dosage of coagulant used in the flocculation assays for each of the initial turbidities (0.125 g/L to 2g/L kaolin).

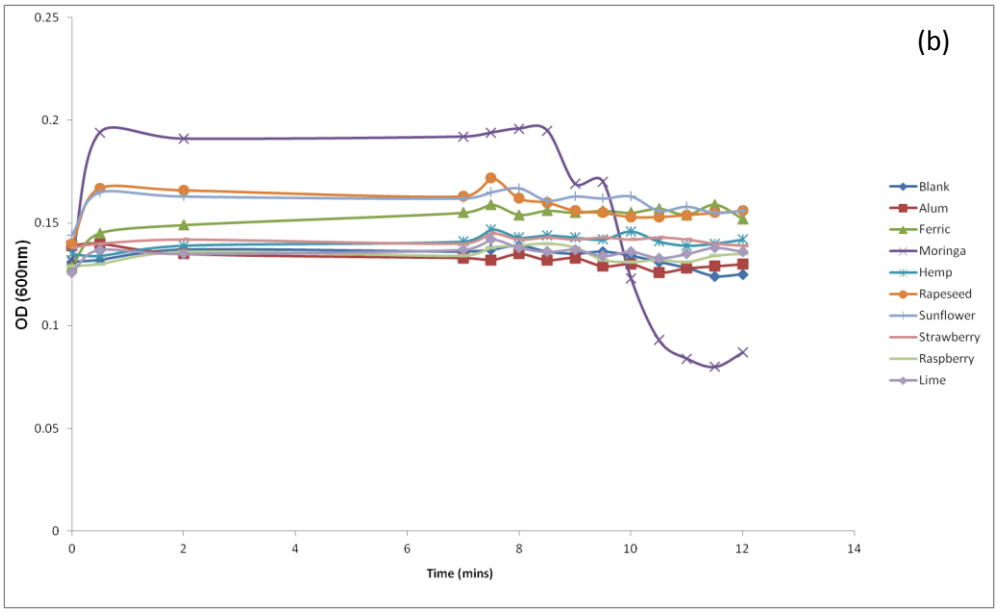
Kaolin	Alum	Ferric	Moringa	Hemp	Rapeseed	Sunflower
	Dosage ppm	Dosage ppm	Dosage ppm	Dosage ppm	Dosage ppm	Dosage ppm
0.125g/L	80	60	80	80	80	40
0.25g/L	40	20	80	80	80	20
0.5g/L	80	20	60	80	80	60
1g/L	20	80	40	20	20	20
2g/L	40	80	20	20	20	40

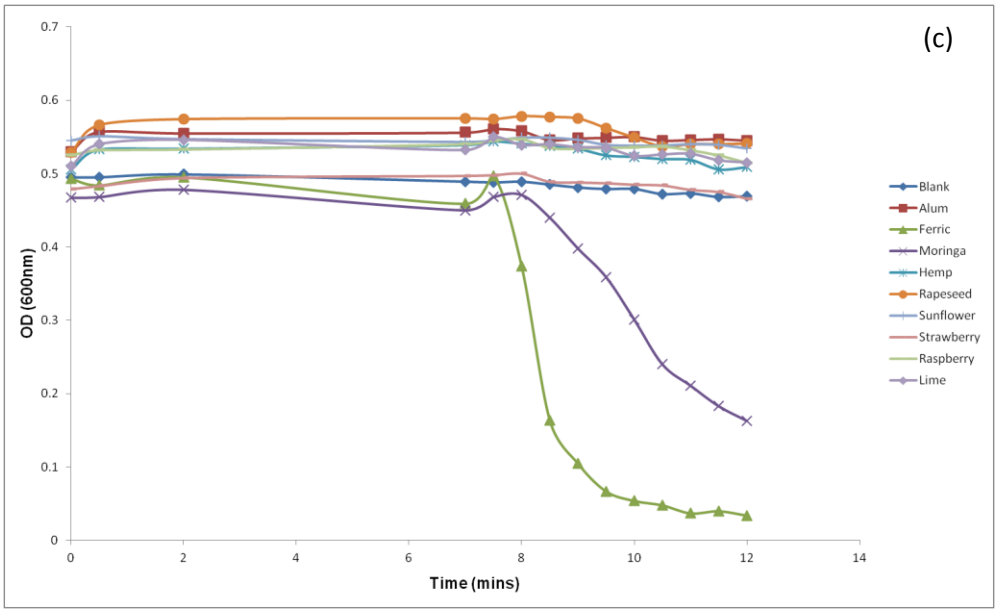
Table 2 summarises the optimum dosage found for each of the press-cake extracts, in each of the initial turbidities (0.125-2g/L Kaolin), based in the initial flocculation rate and %ΔOD between the initial and residual turbidity after cessation of stirring. It is important to consider both factors when optimising the flocculant dosage since a high initial rate and low residual turbidity would be optimum, with activities comparable to alum and ferric, while avoiding increasing the biological, oxygen demand (BOD) of the water to be treated. The results from Figure 2 (a) and (b) and Table 2 show the optimum amount of protein in the assay, over a number of initial turbidities. It also shows that the coagulant dosage varies as a function of the extract. For low initial turbidities, a higher protein concentration (60-80 ppm) was required for all press-cake extracts, alum and ferric. As the initial turbidity was increased to 2 g/L of kaolin, the amount of flocculant required decreased to 20-40 ppm of flocculant. However, there is clearly an optimum level of flocculant, above which no improvement of activity is observed and indeed, beyond which, the residual turbidity actually increases. These results are in agreement with Lamer *et al*., and Muyibi *et al*., in which they postulate the formation of polymer bridges between particles and flocculant, in which excess flocculant can lead to saturation of the polymer bridge sites (Lamer and Healy, 1963; Muyibi and Evison, 1995). An excess of cationic polyelectrolytes such as *M. oleifera* extracts may actually lead to charge reversal and re-stabilization of the particles resulting in too few particles to form inter-particle bridges (Lamer and Healy, 1963; Muyibi and Evison, 1995). This result also highlights the importance of a quantitative jar test method in order to optimise flocculant addition as a function of conditions, since increasing the

concentration of flocculant does not necessarily result in increased flocculation activity. All results from this point forward are based on the optimum dosages found.

2.3.4. Flocculation activity of press-cake extracts, alum and ferric sulphate



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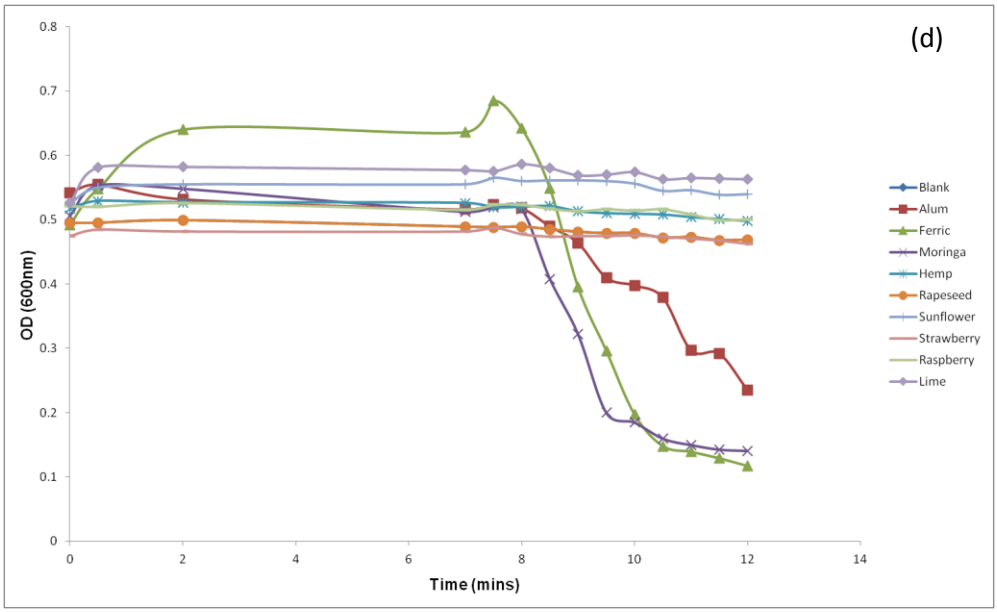
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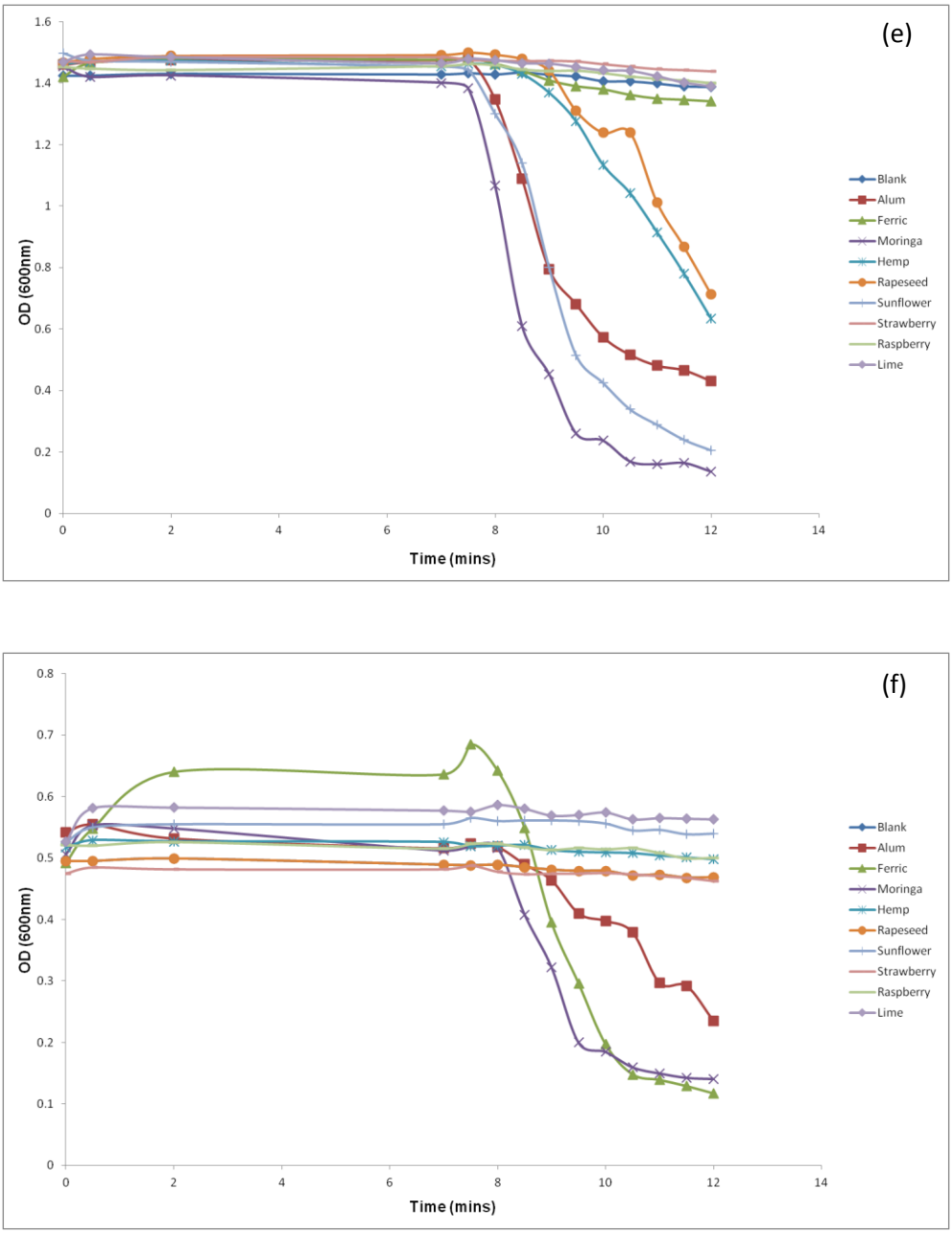


Figure 3. Flocculation activity of press-cake extracts by means of measuring the reduction in OD over a five min sedimentation time after mixing is ceased. (a) 20 ppm flocculant protein, 0.125 g/L kaolin ; (b) 80ppm flocculant protein, 0.125g/L kaolin; (c) 20ppm flocculant protein, 0.5 g/L kaolin; (d) 80ppm flocculant protein, 0.5g/L kaolin; (e) 20ppm flocculant protein, 2g/L kaolin; (f) 80ppm flocculant protein, 2 g/L kaolin.

Table 3. Flocculation activity for the optimum protein concentration by means of measuring the reduction in OD over a five min sedimentation time after mixing is ceased (ppm) (Table2) for all flocculants over a range of initial turbidities with respect to flocculation rate (FR; OD units/min), ΔOD

	Alum			**Ferric**			**Moringa**			**Hemp**			**Rapeseed**			**Sunflower**		
Kaolin	**FR**	**ΔOD**	**% ΔOD**	**FR**	**ΔOD**	**% ΔOD**	**FR**	**ΔOD**	**% ΔOD**	**FR**	**ΔOD**	**% ΔOD**	**FR**	**ΔOD**	**% ΔOD**	**FR**	**ΔOD**	**% ΔOD**
0.125g/L	0.00	0.01	6.47	-0.01	0.07	53.28	-0.03	0.05	34.09	0.00	-0.01	4.58	0.00	0.01	4.58	0.00	-0.01	0.00
0.25g/L	-0.01	0.01	1.87	-0.13	0.46	93.10	-0.05	0.20	76.56	-0.01	0.02	7.09	-0.01	0.02	7.09	0.00	0.01	2.17
0.5g/L	-0.06	0.31	56.64	-0.13	0.46	93.10	-0.09	0.38	65.10	-0.01	0.03	3.68	-0.01	0.03	5.25	-0.01	0.00	0.36
1g/L	-0.20	0.84	87.30	-0.21	0.88	96.16	-0.18	0.79	84.72	-0.03	0.15	16.67	-0.03	0.12	12.50	-0.11	0.56	59.49
2g/L	-0.30	1.29	88.32	-0.32	1.33	93.20	-0.31	1.32	90.65	-0.19	0.83	56.63	-0.18	0.75	51.33	-0.29	1.28	87.73

Figure 3a to 3f and Table 3; show the results of flocculation activity from press-cake extracts as a function of protein and kaolin concentrations. All extracts exhibited flocculation activity, with the exception of raspberry, strawberry and lime extracts. Of the extracts studied, those from *M. oleifera*, sunflower, rapeseed and hemp gave the highest flocculation rate and provided the lowest residual turbidity. Alum and *M. oleifera* extract exhibited similar properties over the whole range of initial turbidities whereas at the higher turbidity of 2 g/L sunflower and ferric showed a flocculation activity comparable to alum and *M. oleifera*. These results indicate that with highly turbid waters *M. oleifera* and sunflower extracts show the highest flocculation rate and lowest residual turbidity, with a flocculation activity comparable to alum and ferric.

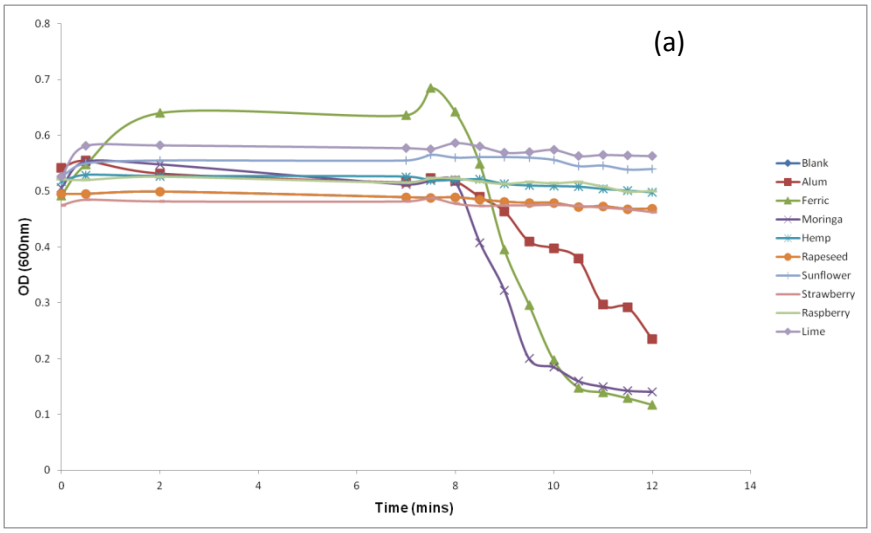
Hemp and rapeseed extracts demonstrated flocculation activity only at turbidities greater than 0.5 g/L of kaolin. The extracts have similar flocculation activity with flocculation rates of 0.18-0.19 OD units/min at high turbidities and a total reduction of between 51 and 56 % in 2g/L kaolin. The lower flocculation activity of hemp and rapeseed compared to *M. oleifera* and sunflower may be due to the flocs having a less robust consistency, which are easily dispersed by the slow mixing occurring during the flocculation assay, with stable flocs capable of sedimentation only appearing after cessation of the mixing.

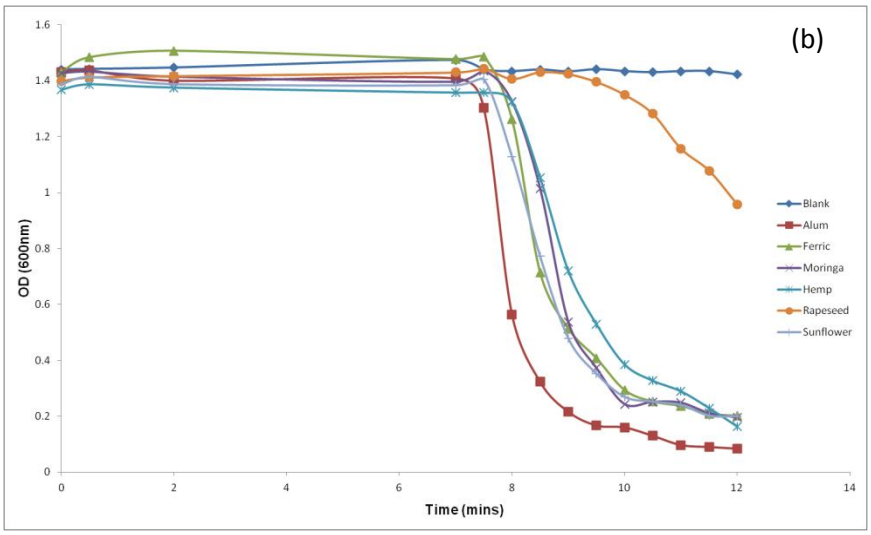
From the results represented in Figures 2a to 2f it can be seen that no flocculation activity was observed with extracts from strawberry, lime and raspberry for all turbidities studied. This suggests that the agent(s) in the press-cake extracts of sunflower, hemp and rapeseed, which are responsible for the flocculation activity, are not present in all oleaginous seed press-cakes. As a result of this preliminary screening, extracts of lime, raspberry and strawberry were not used for further study.

2.3.5. The effect of initial turbidity on flocculation activity

The results from figures 2a to 2f show that for *M. oleifera*, sunflower, hemp and rapeseed extracts, flocculation activity increased with increasing initial turbidity. This is in agreement with Muyibi *et al.* for *M. oleifera* extracts who found that all active flocculants work at their optimum at a higher initial turbidity value (Muyibi and Evison, 1995). Very low, if any, flocculation activity was observed with low kaolin concentrations (<0.5 g/L) even when alum and ferric sulphate were used. The initial turbidity in the jar test has a major influence on the flocculation process since it will change the kinetics of flocculation. High particle concentrations increase the frequency of collisions thereby making flocculation more effective by creating more dense flocs which in turn allows sedimentation to occur at a higher rate. (Guibai and Gregory, 1991) For this reason, in many water treatment facilities particles, such as kaolin or bentonite, are often added to low turbidity solutions to increase flocculation activity. (Guibai and Gregory, 1991)

2.3.6. Effect of pH and ionic strength on flocculation activity




(b)
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Ferric
Moringa
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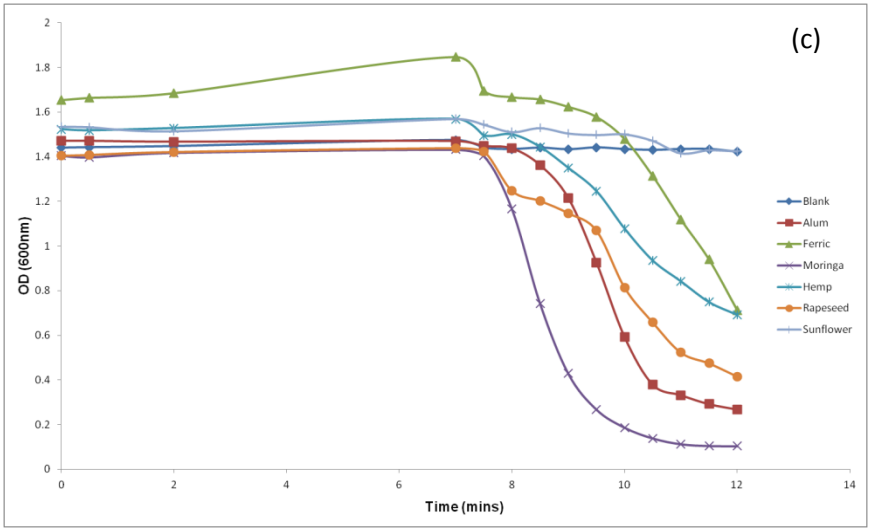
Rapeseed
Sunflower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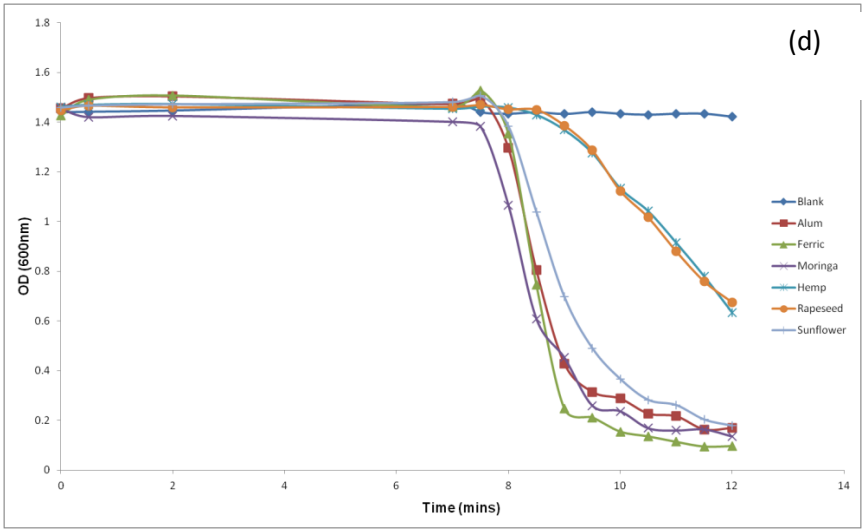
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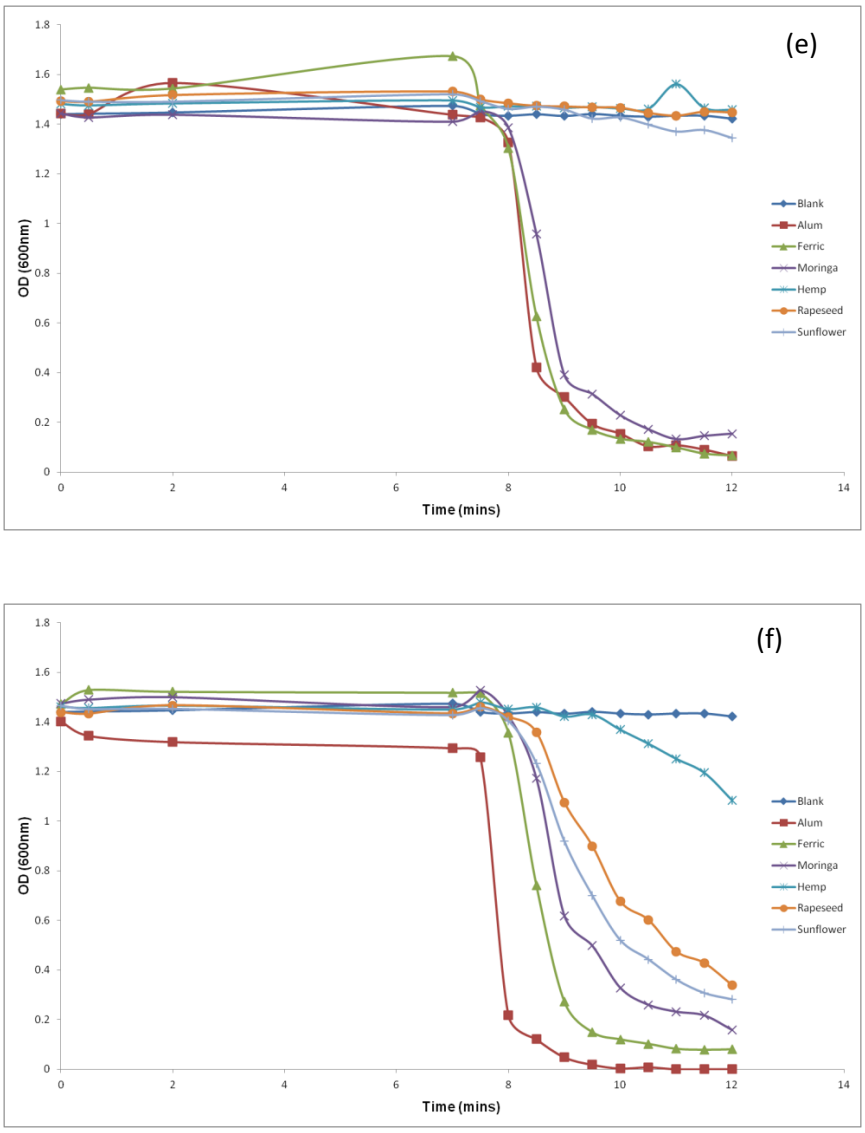


Figure 4. Flocculation activity of press-cake extracts and alum and ferric sulphate by means of measuring the reduction in OD over a five min sedimentation time after mixing is ceased, as a function of pH and ionic strength in 2 g/L kaolin suspensions (a) acetate buffer pH 5, 10mM NaCl ; (b) acetate buffer, pH 5, 100 mM NaCl ; (c) phosphate buffer, pH 7, 10 mM NaCl ; (d) phosphate buffer, pH 7, 100 mM NaCl ; (e) Tris/HCl buffer, pH 9 10 mM NaCl; (f) Tris/HCl buffer, pH 9 100 mM NaCl;.

Table 4. Comparison of flocculation activity at pH 5, 7 and 9 and two ionic strengths 10 mM and 100mM. This data represents the flocculation activity as flocculation rate (FR; OD units/min), change in ΔOD and % ΔOD).

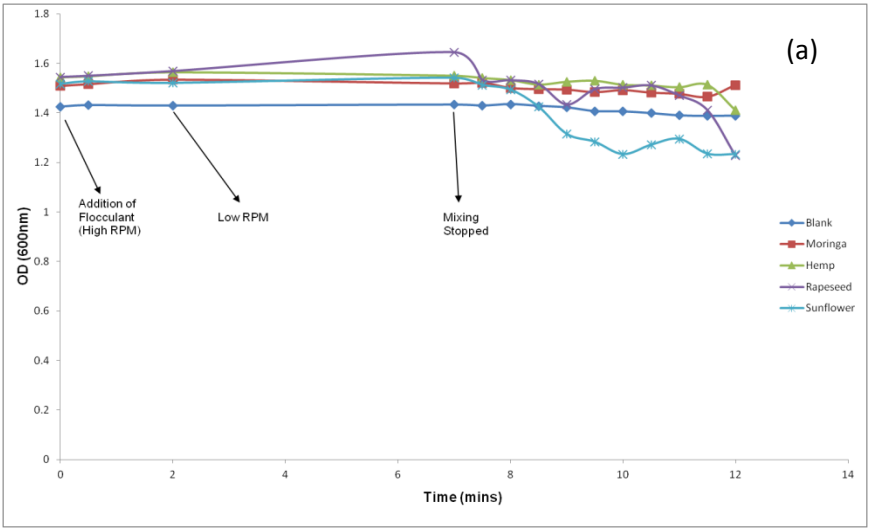
Alum, ferric, *M.oleifera*, hemp extract, rapeseed extract, and sunflower extract were added to a final concentration of 40 ppm, 80 ppm, 20 ppm, 20 ppm,

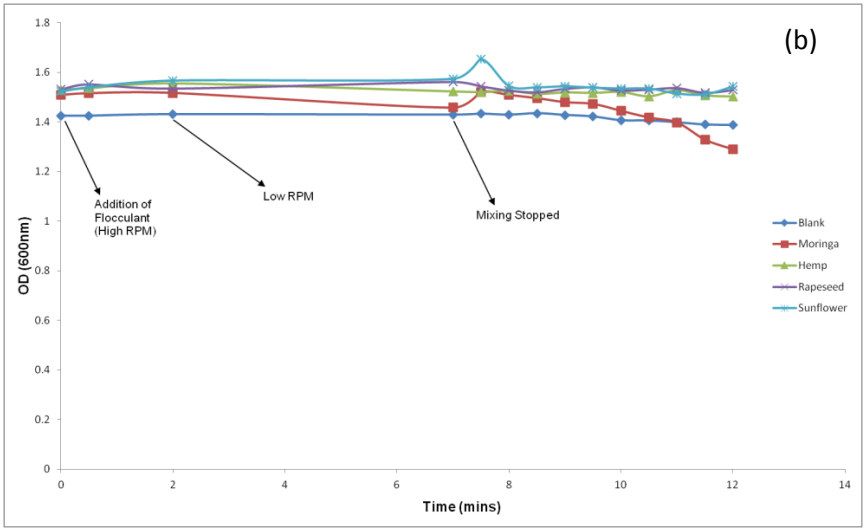
pH	Ionic Strength	Alum			Ferric			Moringa			Hemp			Rapeseed			Sunflower		
		FR	ΔOD	% ΔOD	FR	ΔOD	% ΔOD	FR	ΔOD	% ΔOD	FR	ΔOD	% ΔOD	FR	ΔOD	% ΔOD	FR	ΔOD	% ΔOD
5	10mM	-0.30	1.20	88.90	-0.20	0.94	81.52	-0.30	1.30	97.61	-0.19	0.83	90.31	-0.23	0.99	92.91	-0.03	0.11	80.42
	100mM	-0.30	1.29	94.13	-0.32	1.33	85.95	-0.27	1.32	86.33	-0.17	0.83	88.08	-0.17	0.77	31.59	-0.31	1.28	85.58
7	10mM	-0.31	1.27	81.78	-0.29	1.23	56.89	-0.29	1.39	92.60	-0.35	1.40	54.56	-0.33	1.47	70.37	-0.26	1.15	7.17
	100mM	-0.24	1.35	88.32	-0.29	1.23	93.20	-0.29	1.23	90.65	-0.28	1.21	56.63	-0.09	0.44	53.31	-0.27	1.19	87.73
9	10mM	-0.30	1.38	95.50	-0.33	1.47	95.71	-0.31	1.29	89.27	0.00	0.02	1.62	-0.02	0.05	3.01	-0.03	0.15	10.15
	100mM	-0.23	1.40	100.00	-0.33	1.39	94.57	-0.32	1.32	89.28	-0.07	0.38	25.79	-0.26	1.10	76.51	-0.28	1.19	80.83

pH not only has an influence on the net charge of the flocculant, but also on the particles within the water, and is consequently a key parameter (Letterman and Vanderbrook, 1983; Mietta *et al*., 2009) for assessing the flocculation activity of a target compound i.e. the flocculant under investigation. The ionic strength in water may vary considerably, however high ionic strength generally favours the destabilisation of colloids resulting in their natural settlement whereas a very low ionic strength may provoke the precipitation of *M. oleifera* extracts and yield flocculation activity (Franceschi *et al*., 2002; Guibai and Gregory, 1991). The results from Figures 4a to 4f show flocculation activity with alum and ferric sulphate and the extracts from *M.oleifera*, hemp, rapeseed and sunflower as a function of pH and ionic strength. The amount of flocculant added to the assay, was the optimum level determined from Table 2.

There are interesting variations between the optimum pH profiles and ionic strength for the different flocculants. In the case of alum there was little variation of flocculation activity as a function of pH or ionic strength, although the highest ΔOD was generally obtained with the highest NaCl concentrations. For ferric sulphate there was no such effect with the activity and ΔOD relatively unaffected by pH and ionic strength and the results were very similar to those obtained for *M.oleifera* extracts. Sunflower extracts showed a distinct optimum activity at pH 7 and in the presence of 100 mM NaCl. At pH values below and above 7 the flocculation activity was at least 10-fold lower, with a 10-fold higher activity in the presence of 100 mM NaCl except at pH 7. For hemp and rapeseed extracts the situation was more complex with ionic strength having little effect on the activity or ΔOD of hemp extracts except at pH 9, whereas for rapeseed no clear correlation between ionic strength and pH on the activity could be discerned. However, in all cases it was evident that a high flocculation activity, comparable with alum and ferric sulphate was observed at some pH values and NaCl concentrations. These results suggest that while there are indeed flocculation agents within all extracts, the activity and ability to produce flocs with enough density to sediment in a reasonable time-frame (5 min) was variable, suggesting that the components in each extract are not identical. This offers the possibility of isolating extracts from different oleaginous seed press-cakes which have an optimum flocculation activity under different environmental conditions.

2.3.7. Effect of protein denaturation on flocculation activity





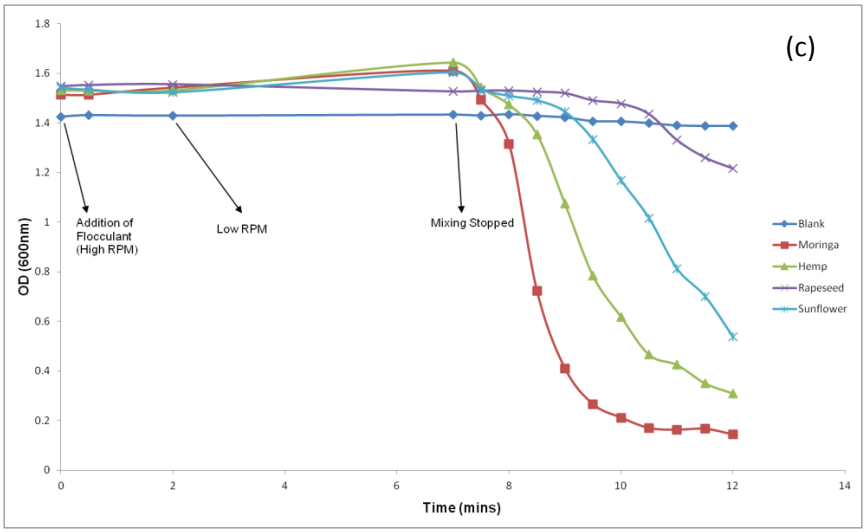


Figure 5. Flocculation activity of press-cake extracts by means of measuring the reduction in OD over a five min sedimentation time after mixing is ceased in 2 g/L kaolin suspensions after treatment with (a) DTT, (b) proteinase K and (c) boiled to 100ºC.

While the ground seeds and press-cake extracts of *M. oleifera* were expected to possess flocculation activity, there has been some debate as to whether the active component(s) are proteins or a small molecular weight species (Gassenschmidt *et al*., 1995; Ndabigengesere *et al*., 1995; Okuda *et al*., 2001b). In order to shed light on this, the extracts from *M.oleifera*, sunflower, rapeseed and hemp press-cakes were treated with a strong reducing agent (0.1M DTT), a protease enzyme (protease K) or boiled to 100ºC . No flocculation activity was observed for any of the extracts after DTT and protease treatment. These results strongly suggest that proteins are indeed responsible for the flocculation activity and that, with the exception of sunflower extracts, disulphide bonds may be involved in the structure of the extract proteins. However, after the extracts were boiled to 100 ºC, flocculation activity appeared unaffected for *M.oleifera* and sunflower with both giving similar FR and □OD values. Unpredictably, results for hemp increased after boiling giving FR of -0.3058 and a reduction in turbidity of 79.8% compared to FR of -0.1929 and 56.63% prior to boiling. This shows that heating of extracts has variable effects, suggesting that the conformation of the proteins is not always essential for activity, with small protein precipitates possibly acting as nucleation sites for initiation of flocculation.

2.4. Conclusions

In this study we have obtained protein-containing extracts from a range of oil seed press-cakes, using a simple standardised salt extraction method. The protein content of these extracts was highly variable, ranging from 16-68 g/L depending on the plant

species. As expected *M.oleifera* extracts showed a high flocculation activity, however three other extracts (sunflower, rapeseed and hemp) also showed high levels of flocculation activity in a standardised, quantitative, flocculation assay. This unique result suggests that the seeds from oleaginous plants contain a range of proteins which exhibit flocculation activity rather than the well-known cationic proteins characteristic of *M. oleifera* seeds. This result, suggests that biological flocculants should be found in a broad range of such plant seeds and their associated press-cakes, and that these extracts might be capable of replacing the currently used salts of iron and aluminium. It is clear that the proteins present in these extracts were responsible for the flocculation activity, and that the extracts from each source showed a different optimum condition of pH and ionic strength, as well as showing different sensitivities to boiling, reducing agents and Proteinase K. This reinforces the idea that a family of proteins are present rather than a single one, and offers the potential to generate a palette of protein extracts in a simple, cheap and environmentally friendly way which may be used individually or combined to get a high flocculation activity and level of turbidity reduction. Unlike *M. oleifera* which only grows in sub-tropical climates, the protein extracts investigated here could be suitable for use in range of climates found in developing countries as well as developed ones. Since the dosage levels of the protein extracts is very small, in the order of 20-40 ppm, they should have a negligible effect on the BOD of the water to which they are added. Furthermore, the absence of alum, ferric sulphate and polymeric flocculants such as acrylamide derivatives, means that the sludge resulting from waters treated with these extracts should not require incineration, but can be broken down simply through biological degradation, resulting in significant cost savings and reducing the environmental load. Further work is required to define how the extracts may be added to, and distributed in, the water treatment process.

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Chapter 3

Application of turbidity meters for the on-line quantitative analysis of flocculation and sedimentation in the Jar Test apparatus

3.1. Introduction

The American Public Health Association (APHA) defines turbidity as "expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample" (American Public Health Association, 1985). Turbidity is an important water quality indicator as it does not only indicate the presence of suspended solids but also shows the possible presence of algae, microorganisms, organic matter and other micro particulates (Environmental Protection Agency Ireland, 2002). Suspended solids in drinking water can often support the growth of harmful microorganisms and reduce the effectiveness of chlorination, therefore making the removal of turbidity a vital process in all water treatment plants (Barron, 2005). Coagulation and flocculation are key steps in water treatment as these are the most effective ways of removing turbidity from water. This involves the removal of suspended micro particulate matter by the addition of highly charged cations such as aluminium and iron salts, usually in the form of aluminium sulphate ($Al_2(SO_4)_3$) and ferric sulphate ($Fe_2(SO_4)_3$), commonly termed alum and ferric sulphate respectively, to water (Diaz *et al*., 1999; Pritchard *et al*., 2010; Rossini *et al*., 1999). These ions neutralise the electrical charges on the suspended solids and colloids which in turn form flocs. The flocs grow in size and merge and due to the increased mass and density, cause sedimentation which can ease their removal by filtration or clarification (Barbot *et al*., 2010; Cape Canaveral, 1996). The world health organisation (Word Health Organisation, 2007) states that water, to be disinfected, turbidity levels must be <1.0NTU.

Turbidity is an optical property of the interaction between light and suspended solids in water, allowing light to be scattered and absorbed rather than transmitted through straight lines (Gentile *et al*., 2010; Grayson *et al*., 1996). When a beam of light is passed through ultra-pure water the light path remains relatively undisturbed. However, particulates (suspended solids) will interfere with the light beam and absorb the light energy and/or scatter or reflect the light (Sader, 1998). In turbidity probes, the intensity of the scattered light increases with suspended solids. The scattering is reflected at an angle of 180° and then is captured and led back via a fibre optic cable to a photodiode and processed as photocurrent and the signal transformed into a value (NTU) for the transmitter (Gentile *et al*., 2010; Sader 1998).

Effective flocculant dosage is mainly determined by the results of the standard jar test method. If inaccurate measurement of flocculation activity is calculated this can often lead to wasteful over dosing of chemical flocculants in water treatment plants causing greater operation costs and increased chemical sludge (Cheng *et al*., 2008; Franceschi *et al*., 2002). Accurate measurement of flocculation activity can often be difficult as samples need to be taken off line and inserted to into a turbidimeter or spectrophotometer, making these methods extremely time consuming and inaccurate. This has led to the development of on-line turbidity meters to be attached to the standard jar test apparatus. To date there are few online methods that focus on measuring floc size by image analysis and microscopy (Cheng *et al*., 2008; Cheng *et al*., 2010; Tse *et al*., 2011), however these methods tell us very little about the flocculation sedimentation rates and more about floc formation. Flocculation sedimentation rates and residual turbidity values are vital to any water treatment plant process, as they tell the user how efficient the flocculant is for the required water at any given time (Tse *et al*., 2011).

Therefore the main aim of this section of the project was to develop and validate a new on-line quantitative process for the analysis of flocculation activity by measuring the sedimentation rate and change in turbidity from initial turbidity to final turbidity. This was achieved by attaching six Mettler Toledo Turbidity Transmitter and TRB 8300 probes to a data acquisition (Lab View), each of the probes were then be placed inside the vessels in the Phipps and Bird PB-700 Jar Test

apparatus. This will allow six online measurements of flocculation and sedimentation activity to be measured at once rather than the original single Jar test assay. The first section of the setup of the probes was to complete an instrument calibration, in order to determine that the six Mettler Toledo Turbidity Transmitter TRB 8300 probes all worked in the same manner and the process was reproducible between each probe. This was completed by assessing capabilities of the instruments through experiments such as, multipoint calibration, limit of detection, and sensitivity, testing the interfering light and flocculant colouring effects on the readings from the probes. Once the results had proven that all probes work within a 95% confidence interval for the measurement of the flocculation assay, a process validation was complete. In order to complete this process validation the standard flocculation chemicals that are used in many water treatment plants (aluminium sulphate (8% w/v) and ferric sulphate (11.7%)) was used in the flocculation assay. Results show that the Phipps and Bird PB-700 standard jar tester and the six Mettler Toledo INPro 8000series turbidity probes setup, are capable of analysing the flocculation rate and change in turbidity within 95% confidence intervals. This will aid the efficiency and accuracy of the experimentation by allowing six individual online measurements of flocculation and sedimentation activity to be measured in parallel at once rather than the original single Jar test assay.

3.2 Materials and Methods

3.2.1 Instrumentation Set-up

To a Phipps and Bird PB-700 standard jar tester, six Mettler Toledo INPro 8000series turbidity probes were attached in order to give an online measurement of flocculation and sedimentation. The probes were placed in the top of the beakers. The transmitters are equipped with a light emitting diode (LED) in the almost infra-red range (880 nm) via a fibre optic cable. The probes work on the basis of backscattered light; the light is scattered at an angle of 180°, is captured and led back via a fibre optic cable to a photodiode to the Mettler Toledo turbidity transmitters (Trb 8300) and processed as photocurrent and the signal transformed into a value

(NTU) for the transmitter. All six turbidity transmitters are connected to the data acquisition system, Lab View, by the means of RS232 cards.

The probes were carefully positioned to the optimum height and distance from the vessel wall and propeller. This was be completed by positioning the probe at varying heights, whilst keeping the probe at a constant distance from the vessel wall. Once the optimum height was determined, the distance from the vessel wall was calculated by keeping the height of the probe constant and adjusting the probe distance from the vessel wall. The height optimised to 80 mm from the base of the vessel and the distance was optimised to 25 mm from the vessel wall, this that gives the correct turbidity value was taken as the optimum position of the probe. The probes were attached in this position for the remainder of the experiments.

3.2.2 Instrumentation Calibration

The six probes were calibrated via a multipoint calibration. This multipoint calibration is used when a solution with a known turbidity value, therefore a set of standard Formazin Suspensions (Hach Lange, Ireland) with a turbidity value of 2000, 1000, 500, 100, and 20 Nephelopmetric Units (NTU) were used to calibrate each instrument.

The calibration was carried out by the following procedure:
The sensor was dipped into the sample with the highest concentration first. The known measured value of the sample was entered and then the <<Page Down>> on the transmitter was pressed. A message indicating that the calibration is in progress was displayed. The sensor was rinsed with clear solvent (< 0.5% HCL) and the calibration proceeded to the second point with the second highest concentration. This procedure was continued until all the concentrations were completed. Once the multipoint calibration was completed the transmitter will goes back to the measuring mode automatically using the new calibration data.

3.2.3 Interfering light

Synthetic turbid waters were prepared by the addition of kaolin (Fisher Scientific U.K.) to deionised water until the desired initial turbidity was achieved. High particle concentrations increase the frequency of collisions thereby making flocculation more effective, whereas interfering light will have a greater effect especially on water with a low turbidity value. In order to determine the effect of particle concentration and interfering light, the NTU of a series of suspensions containing 2 g/L, 1 g/L, 0.5 g/L, 0.250 g/L and 0.125 g/L of kaolin were measured. The kaolin suspensions were incubated for 24 h., at room temperature, with gentle stirring to allow for complete hydration of the kaolin, prior to use.

Firstly, the probes were calibrated using formazin suspensions from 20 to 2000 NTU. The probes were then tested using five different kaolin concentrations from 0.125 g/L to 2 g/L. The first run was in completely darkened conditions using black card in order to eliminate any outside natural light and the second conditions were partially dark conditions i.e. the front of the beakers was exposed to natural ambient light. The suspensions were left to mix at a high RPM for 2 minutes and a low rpm for 2 minutes. The end result of this experimentation will show if any interfering outside light has an effect on the output from the probes. To establish if the data was normally distributed a statistical method for normal distribution, Shapiro-Wilk was used. If the data is normally distributed, this shows that these are the optimum conditions for using the turbidity probes.

3.2.4 Reproducibility

Reproducibility between probes is highly important as it will determine that the calibration applied to one probe can also be applied to all five other probes. This will then determine whether all six probes will give out the same value for the same sample.

To establish repeatability between the 6 probes and within the probes themselves, a test is done with 100 and 1000NTU formazin suspensions for instrumentation

calibration and kaolin suspensions 0.125g/L to 2g/L for process validation was completed. The suspensions were stirred 5 minutes before the reading to ensure complete mixing of the Kaolin before recording results. The data was then analysed to determine the percentage error of each turbidity meter. The 95% confidence interval of the offset within the turbidity probes was determined.

3.2.5 Sensitivity and Limit of detection (LOD)

To 1 litre beaker of deionised water 0.05g kaolin was added and allowed to mix at a high RPM for two minutes. 0.05g/L of kaolin was added in two minutes intervals until a final concentration of 2g/L kaolin was achieved and the NTU was determined. This procedure was repeated three times. From these results the sensitivity of the turbidity meters can be detected and a NTU value for kaolin concentration was also calculated. The limit of detection (Jones *et al*., 2003) was calculated based on calibration data and regression statistics were the *y*-intercept and standard deviation of the regression is used:

$$YLOD = regression + 3*STDEV$$

Where the regression is the regression of the line and 3*STDEV is the standard deviation of y-intercepts of regression lines.

3.2.6 Colour of flocculants

As coagulants such as ferric sulphate often have a colouring effect on water and suspended particles it is important so see if the coagulants have an effect on the readings of the probes. Alum and ferric sulphate were added to 1 L of deionised water from 0 to 100 ppm in 5 ppm intervals. This was repeated in kaolin suspensions 0.125, 1 and 2 g/L. For the effect of colouring on the kaolin suspensions a Two Way - Analysis of Variance (ANOVA) statistics shows the interaction between the

coagulants and the deionised water and kaolin solutions. (McDonald, 2009) This method enables the means of two or more groups to be compared, in response to two different independent variables, i.e. concentration of kaolin and volume of flocculant.

3.2.7 Process Validation

From the LOD and the effect of interfering light studies, the probes were calibrated in darkened conditions using kaolin suspensions of 0, 0.125, 0.25, 0.5, 1 and 2 g/L. In order to validate this calibration for the process of using kaolin as a calibration solution, a repeatability study was completed. Reproducibility between probes is highly important as it will determine that the calibration applied to one probe can also be applied to all five other probes. This will then determine whether all six probes will give out the same value for the same sample.

The data was then analysed to determine the percentage error of each meter. This will determine the 95% confidence interval and the offset within the turbidity probes. The repeatability between the 6 probes and for each probe was investigated. This test is done using 0.125g/L to 2g/L of kaolin. The suspensions were stirred 5 minutes before the reading to ensure complete mixing of the kaolin before recording results for a two minute period.

3.2.8 Flocculation assay

In order to test the turbidity probes for measurement of flocculation activity a series of flocculation assays were performed in order to quantify sedimentation rate and change in NTU. To do this, a jar test procedure was set up using 6 one litre kaolin suspensions with the required initial turbidity (0.125 to 2 g/L) placed in beakers together with the required volume of flocculant, (alum or ferric sulphate). The suspension was mixed at a high rate (200 rpm) for 2 minutes followed by a low

mixing rate (50 rpm) for 5 minutes and finally no mixing for 5 minutes (Acorn water, Ireland). Continuous on-line measurement readings were taken using the data acquisition system (Lab View). The Turbidity transmitters connected to Lab View via RS232 communications, the data is transferred in 3 second intervals and the data is pre-processed in 15 s M.P.A in order to reduce noise.

The sedimentation rate was calculated by determining the initial slope (7 to 10 minutes) of the turbidity readings (NTU). The total level of sedimentation was determined from the turbidity value of the kaolin solution after addition of flocculant minus the turbidity value at 12 min after addition of flocculant. The rate of flocculation was used to determine which flocculants formed flocs at the highest rate, whereas the sedimentation level was used to determine the density of the flocs and the ability to sediment within 12 min.

3.3 Results and Discussion

3.3.1 Effect of Interfering light and normality of data

Normality is one of the most common assumptions made in the development and use of statistical procedures. The normality of an underlying data distribution can have a considerable effect on the properties of estimation or inferential procedures used in the analysis of the data (Kim, 2011). In order to prove that the data from the six turbidity probes is normally distributed over a low turbidity of 100 NTU and a high turbidity of 1000 NTU a test for normality was completed using SPSS (Statistical Package in Social Science).

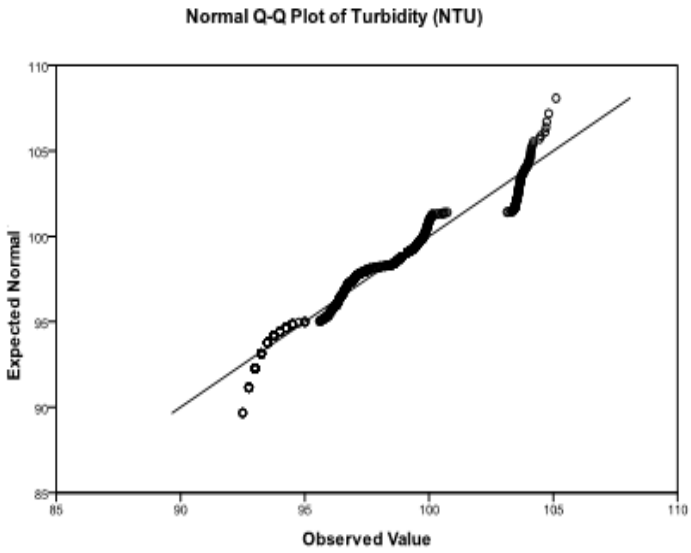

Figure 1. Shapiro-Wilk Q-Q plot where the observed turbidity (NTU) is plotted against the expected turbidity (NTU) values for 100NTU formazin suspensions in natural light conditions. Data is not normally distributed as the points deviate from the straight line.

Table 1. Shapiro-Wilk test of normality data for 100 NTU in natural light conditions

Probe	Shapiro-Wilk		
	Statistic	df	Sig.
1	0856	41	0
2	0.763	41	0
3	0.744	41	0
4	0.992	41	0.989
5	0.764	41	0
6	0.358	41	0

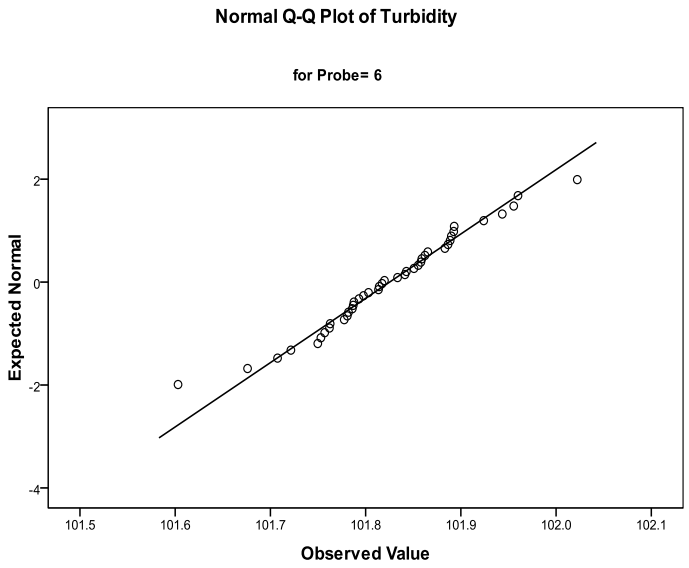

Figure 2. Shapiro-Wilk Q-Q plot where the observed turbidity (NTU) is plotted against the expected turbidity (NTU) value for 100 NTU formazin suspensions in darkened conditions. Data is normally distributed as it follows the diagonal line closely and does not appear to have a non-linear pattern.

Table 2. Shapiro-Wilk test of normality data for 100 NTU in darkened conditions

Probe	Shapiro-Wilk		
	Statistic	df	Sig.
1	0.948	42	0.056
2	0.964	42	0.204
3	0.972	42	0.385
4	0.955	42	0.094
5	0.967	42	0.262
6	0.986	42	0.884

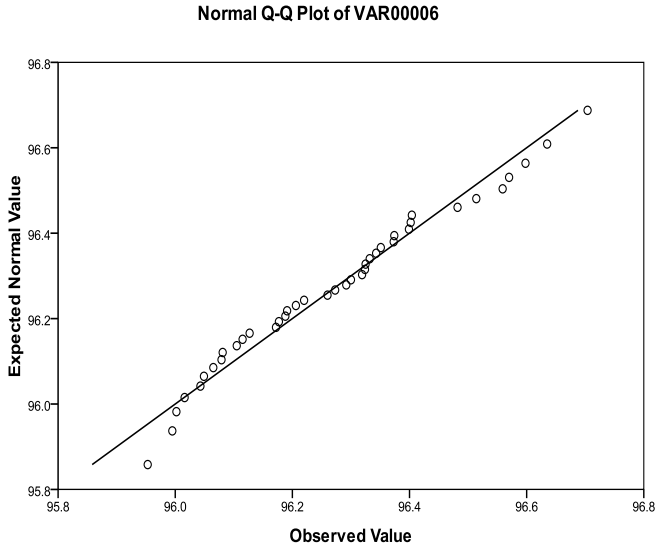

Figure 3. Shapiro-Wilk Q-Q plot where the observed turbidity (NTU) is plotted against the expected turbidity (NTU) value for for 1000 NTU formazin suspensions in darkened conditions. Data is normally distributed.

Table 3. Shapiro-Wilk test of normality data for 1000 NTU in darkened conditions

Probe	Shapiro-Wilk		
	Statistic	df	Sig.
1	0.972	36	0.332
2	0.965	36	0.272
3	0.965	36	0.168
4	0.978	36	0.256
5	0.955	36	0.155
6	0.963	36	0.155

The SPSS gives us two ways of measuring normality, graphically using a normal Q-Q plot and numerically using Shapiro-Wilk. Shapiro-Wilk is based on regression and correlation.

It can be concluded from the normal Q-Q plot of turbidity in Figure 1 that the data in natural light conditions is not normally distributed as the, however, when the vessels are completely darkened by completely enclosing the jars with the kaolin suspensions, the data appears to be normally distributed, as it follows the diagonal line closely and does not appear to have a non-linear pattern. This is further proven by the numerical data presented in Tables 1 to 3. If the Sig. value in the Shapiro-Wilk test is greater than 0.05 then the data is normal. If it is below 0.05, which can be seen from the data in the natural light conditions in Table 1, then the data significantly deviate from a normal distribution. As can be seen in Table 2 and 3, for all six probes in the darkened conditions the Sig. value is greater than 0.05 proving that the data is normally distributed for a low turbidity value of 100 NTU formazin suspension sand a high turbidity value in a 1000 NTU formazin solution. From this result, any experimentation where the probes will be used, all external light will be removed and the Jar Test will be run in darkened conditions.

3.3.2 Repeatability

By confirming that the data within each probe is normally distributed, it was now possible, by the means of descriptive statistics to determine the confidence limits of the six probes. The next test was to show that not only was each probe repeatable but the probes work well within themselves but that all six probes give similar outputs for the same sample.

100NTU

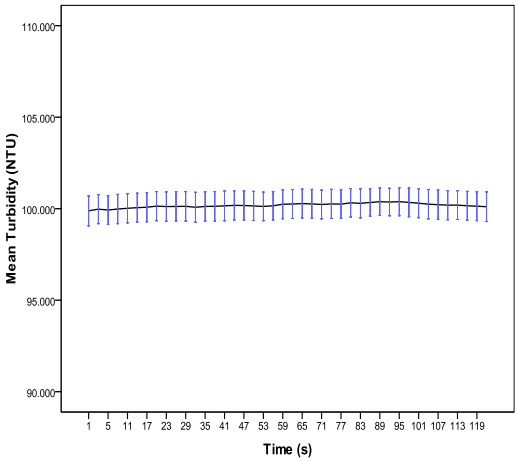

Figure 4. Repeatability measurement of a 100 NTU formazin solution over probes 1 to 6 including 95 % confidence intervals.

1000NTU

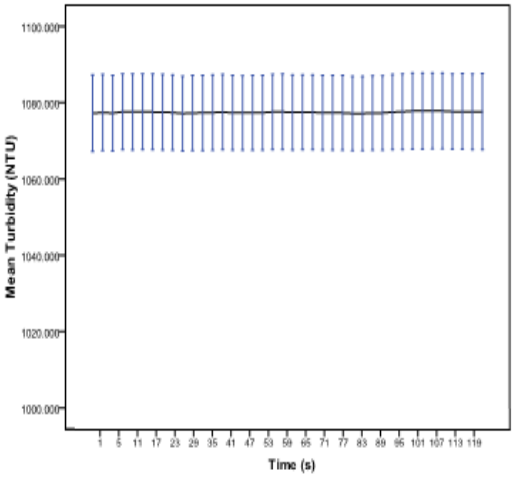

Figure 5. Repeatability measurement of a 1000 NTU formazin solution over probes 1 to 6 including 95 % confidence

Table 4. Statistical data for the repeatability (measured in triplicate) of a 100 NTU formazin solution for probes 1 to 6

	Probe 1	**Probe 2**	**Probe 3**	**Probe 4**	**Probe 5**	**Probe 6**
Mean	99.906	102.425	98.601	98.466	99.864	101.878
Confidence, 95%	0.127	0.040	0.070	0.035	0.045	0.023
STDEV	0.723	0.226	0.399	0.199	0.256	0.133
Deviation from pop. mean	0.283	2.231	1.586	1.721	0.326	1.685
STD error	0.065	0.020	0.036	0.018	0.023	0.012

Table 5. Statistical data for the repeatability (measured in triplicate) of a 100 NTU formazin solution for probes 1 to 6

	Probe 1	**Probe 2**	**Probe 3**	**Probe 4**	**Probe 5**	**Probe 6**
Mean	1002.92	1004.63	1007.74	998.216	1003.3	1001.11
Confidence, 95%	0.182	0.134	0.116	0.071	0.062	0.08
STDEV	1.003	0.74	0.64	0.389	0.341	0.441
Deviation from pop. mean	0.007	0.164	0.474	0.476	0.032	0.187
STD error	0.093	0.068	0.059	0.036	0.032	0.041

Figures 4 and 5 along with Tables 4 and Table 5 showing repeatability statistics for a 100 and 1000 NTU formazin suspensions respectively measured in triplicate using probes 1 to 6. This data shows that for the standard low and high turbidity suspensions that that the all probes read within 95% confidence interval for suspensions with the same NTU of any sample within the calibrated range. From these results it was proven that the instruments are validated and that for the standard turbidity suspensions of formazin they could accurately determine the NTU output.

3.3.3 Instrument Calibration

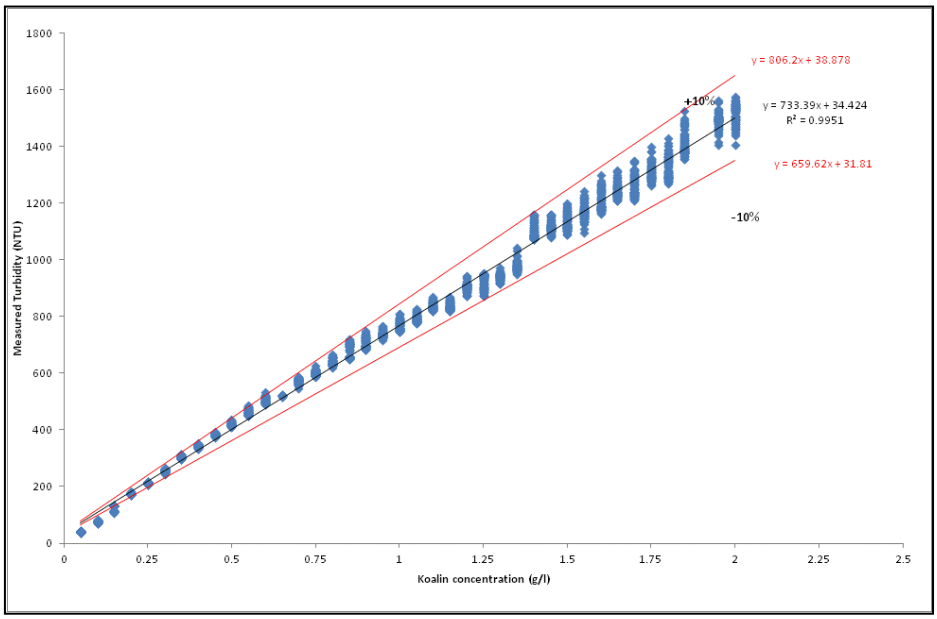

Figure 6. Calibration Curve from 0 to 2 g/L in 0.05g/L. All suspensions were measured in triplicate. The graph shows that for each kaolin suspension each probe reads within ±10%

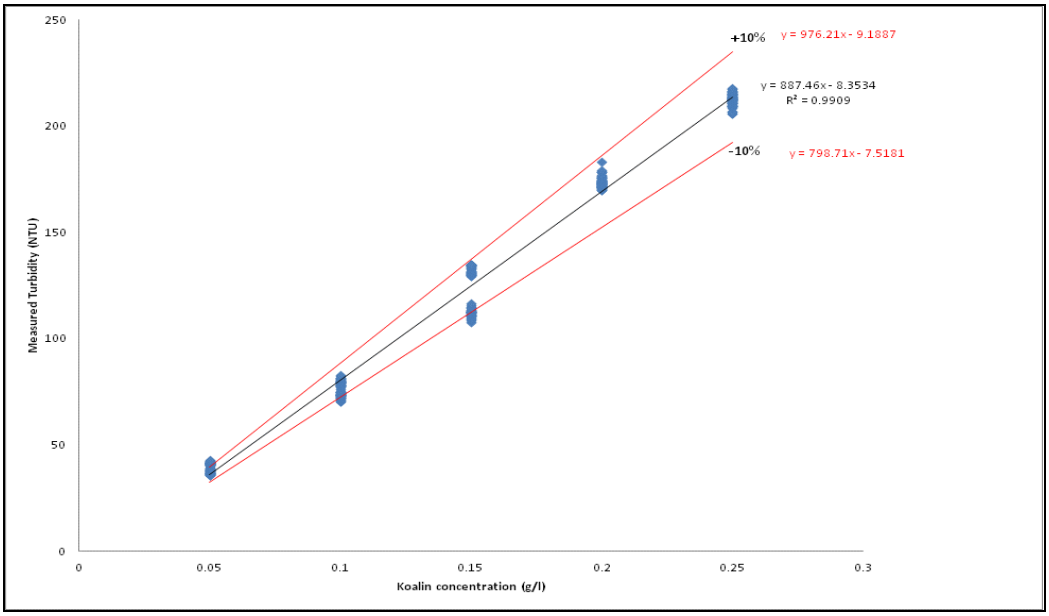

Figure 7. Calibration Curve from 0 to 0.25 g/L in 0.05g/L. All suspensions were measured in triplicate. The graph shows that for each kaolin suspension each probe reads within ±10%

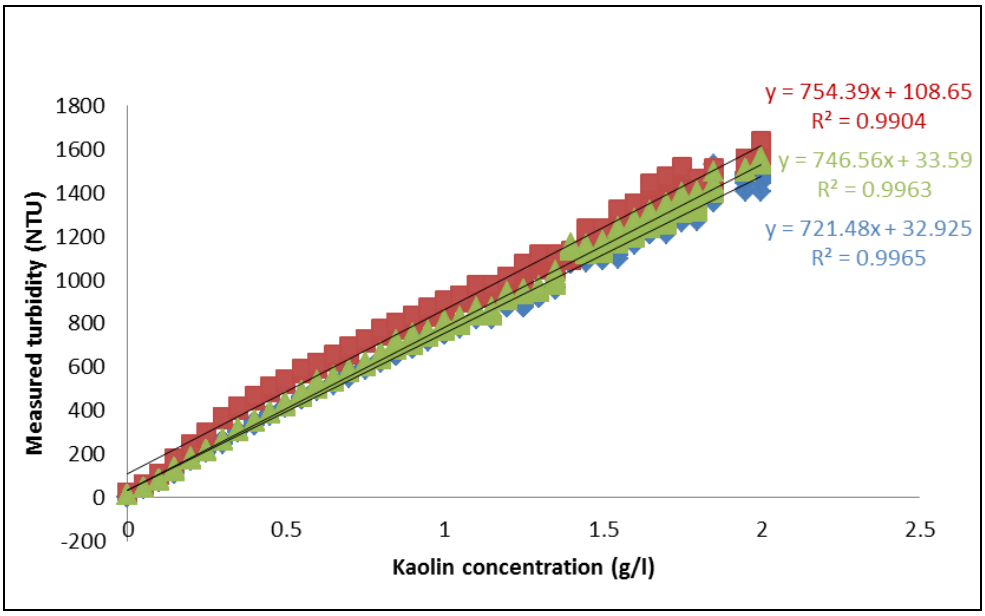

Figure 8. Turbidity response graph for probes 1 -3from 0 to 2 g/L with the addition of kaolin on 0.05 g/L, where blue is probe 1, red is probe 2 and green is probe 3.

Using three of the six probes, a limit of detection and sensitivity study was completed by preparing a set of kaolin suspensions containing 0 g/L and adding 0.05 g/L until a final solution of 2 g/L was achieved. Figure 6 shows a calibration curve from 0 to 2 g/L, following a 5 point multipoint calibration using formazin suspensions from 20 to 2000 NTU. This graph shows that all three probes read within ±10% of each other. Results show that there was some offset between each of the probes, however, this can be overcome by mean anchoring the data. This can be achieved by using the response graph shown in Figure 8 to find the difference of the intercept from the mean data.

From the calibration curve in Figure 6 the limit of detection was calculated as 8.1 NTU and the instrument sensitivity was calculated by means of the slope of the linear regression plot giving a result of 733.39 NTU per g/L. By using this information, the probes were calibrated using kaolin suspensions from 0 to 2 g/L for the purpose of measuring the flocculation assay. Although in figure 6 a linear regression of 0.996 was achieved, on closer examination of this linear regression, the 0 – 0.25 g/L data is not quite linear showing that there may be some error for the

lower turbidity suspensions when the large scale calibration of 0 to 2 g/l was completed. However, when a calibration was completed in the lower range of 0 to 0.5 g/L this greatly improves the linearity of the lower turbidity suspensions which can be seen on Figure 7 which gives on R^2 of 0.995. This result further enhances the requirement for calibrating the process over two scaling ranges of the probes for monitoring flocculation and sedimentation, for low initial turbidity (< 200 NTU) suspensions a multipoint calibration of between 0 and 0.5 g/L and for high turbidity suspensions (between 200 and 1600 NTU) suspensions a multipoint calibration of between 0 and 2 g/L is required in order to achieve optimum operation range.

3.3.4 Process Validation

3.3.4.1 Kaolin Calibration and repeatability

In order to determine if the turbidity probes are suitable for measuring the process of coagulation, flocculation and sedimentation in kaolin solutions, firstly the probes were calibrated and characterised using a series of kaolin solutions. The values for these samples were calculated using a linear regression plot giving a result of 733.39 NTU per g/L. Using this result and multipoint calibration was completed using kaolin suspensions 0.125, 0.25, 0.5, 1 and 2 g/L

Table 6. The Shapiro-Wilk test results for normally distribution of data for the six probes over three runs using a 2 g/L kaolin solution. If the **Sig.** value of the Shapiro-Wilk Test is greater the 0.05 then the data is normal. If it is below 0.05 then the data significantly deviate from a normal distribution.

	Shapiro-Wilk		
	Statistic	df	Sig.
Probe 1 Run1	.932	40	.019
Probe 1 Run 2	.976	40	.543
Probe 1 Run 3	.964	40	.223
Probe 2 Run 1	.969	40	.336
Probe 2 Run 2	.965	40	.257
Probe 2 Run 3	.969	40	.336
Probe 3 Run1	.912	40	.420
Probe 3 Run 2	.950	40	.339
Probe 3 Run 3	.978	40	.615
Probe 4 Run 1	.969	40	.344
Probe 4 Run 2	.969	40	.344
Probe 4 Run 3	.977	40	.583
Probe 5 Run 1	.974	40	.461
Probe 5 Run 2	.987	40	.919
Probe5 Run 3	.992	40	.992
Probe 6 Run1	.986	40	.892
Probe 6 Run 2	.974	40	.479
Probe 6 Run 3	.976	40	.539

Table 7. Shows the statistical results of the repeatability study for all six probes using kaolin suspensions from 0.125/L to 2 g/L

Kaolin		Probe 1	Probe 2	Probe 3	Probe 4	Probe 5	Probe 6
0.125g/L	**Mean**	130.73	213.84	156.24	123.65	102.16	264.33
	Confidence, 95%	0.51	1.05	1.89	1.38	0.22	6.6
	STDEV	2.91	5.93	10.67	7.79	1.24	37.33
	Deviation from pop. Mean (%)	20.85	29.48	5.4	25.13	38.15	60.05
0.25g/L	**Mean**	217.15	351.79	224.79	204.67	286.68	314.83
	Confidence, 95%	0.84	0.39	18.9	0.53	0.62	0.69
	STDEV	4.75	2.19	106.97	2.97	3.52	3.88
	Deviation from pop. Mean (%)	22.19	26.06	19.45	26.66	2.72	12.81
0.5g/L	**Mean**	474.38	604.26	444	646.77	536.28	605.29
	Confidence, 95%	1.72	0.87	2.43	2.43	2.35	2.92
	STDEV	9.75	4.93	13.77	13.73	13.32	16.52
	Deviation from pop. Mean (%)	14.03	9.5	19.54	17.2	2.82	9.69
1g/L	**Mean**	889.07	1003.87	975.37	1098.97	1001.52	1094
	Confidence, 95%	1.89	5.62	3.64	3.24	3.63	3.51
	STDEV	10.69	31.77	20.6	18.32	20.56	19.86
	Deviation from pop. Mean (%)	16.15	5.32	8.01	3.65	5.54	3.18
2g/L	**Mean**	1681.43	1893.03	1863.24	1981.51	1891.17	1890.87
	Confidence, 95%	4.06	2.32	7.07	5.25	4.53	6.97
	STDEV	23	13.13	39.98	29.7	25.66	39.47
	Deviation from pop. Mean (%)	5.32	6.59	4.91	11.57	6.49	6.47

The kaolin calibration was tested using the Shapiro-Wilk method to test that the entire probe data was normally distributed, which can be seen in Table 6. With the exception of probe 1 run 1 which gave a Sig. value > 0.05, this result is most likely down to probe fouling as when this run was repeated in run 2 and 3 the data was found to be normally distributed. Table 7 shows the descriptive statistics following a multipoint calibration. The results from Table 7 show that with increased turbidity the error within the data from the turbidity probes greatly decreased. Low turbidity samples often have a higher level of interference due to stray light, even though for these samples with outside interfering light has been eliminated from the vessels, but there may also be stray light that comes from within the sensor and reflections within the sample cells which cannot be eradicated and this stray light cannot be zeroed out causing low turbid samples to have a much greater percentage error from the population mean than high turbid samples. This can clearly be seen of the percentage error from the population mean for the 0.125 g/L to the 2 g/L results in Table 7; as the kaolin concentration increased the percentage error decreased.

This result proves that for the process of the flocculation assay, the probes can quite accurately be calibrated for the Jar Test process of using kaolin solutions.

3.3.5 Colouring Effect from Coagulants

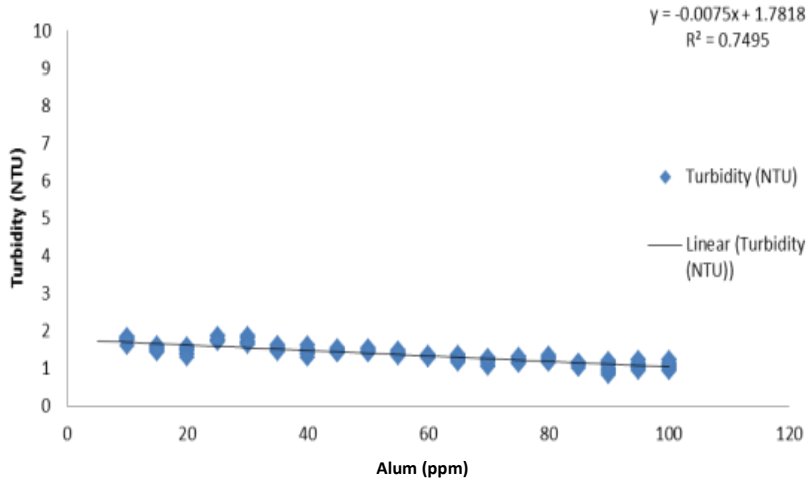

Figure 9. Response graph for the addition of alum from 0 to 100 ppm to deionised water where the turbidity response is measured in NTU

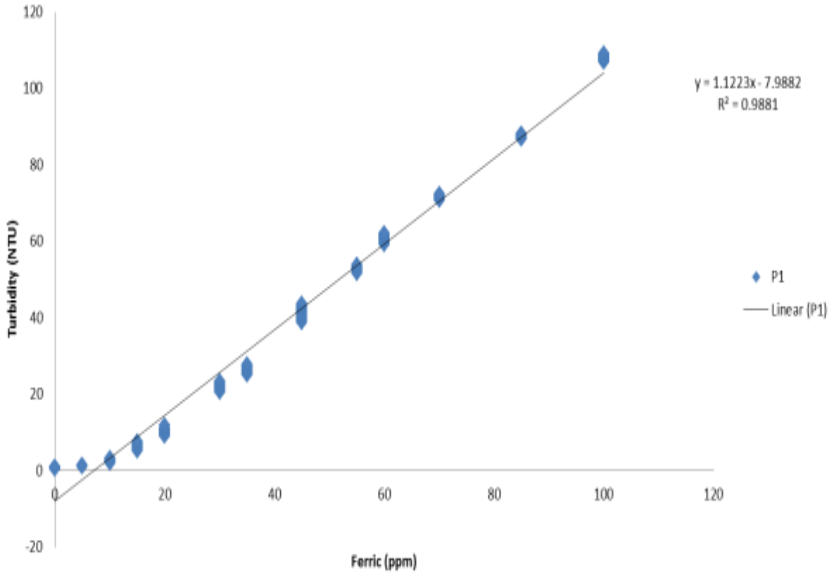

Figure 10. Response graph for the addition of ferric sulphate from 0 to 100 ppm to deionised water where the turbidity response is measured in NTU

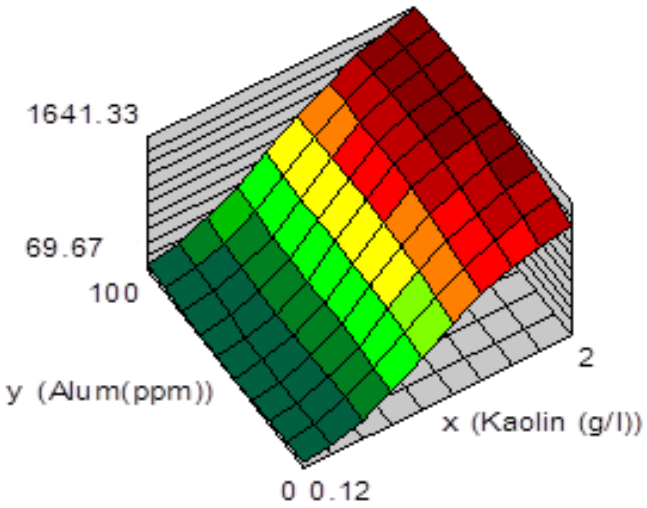

Figure 11. Response surface for measured turbidity with varying alum (0 to 100 ppm) and kaolin concentrations (0.125 to 2 g/L)

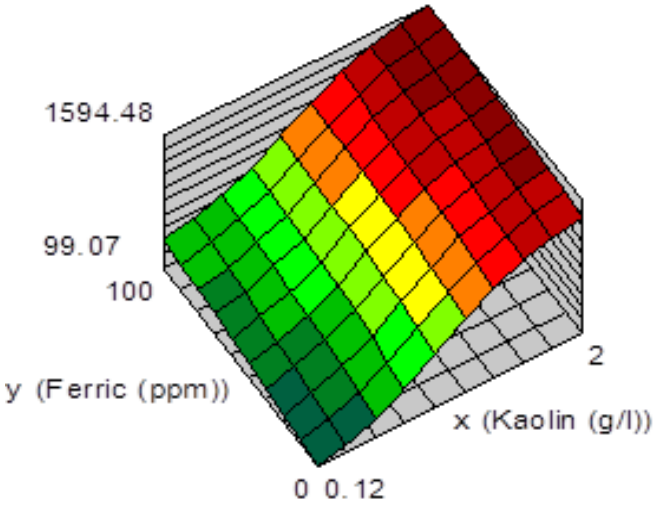

Figure 12. Response surface for measured turbidity with varying ferric sulphate (0 to 100 ppm) and kaolin concentrations (0.125 to 2 g/L)

Table 8. Anova statistics (Analysis of Variance) for ferric sulphate in kaolin solutions

ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-Value*	*F crit*	
Sample	6492796	2	3246398	10007.1	0	3.55	Reject Null Hypothesis
Columns	244141	2	122070	376.284	0	3.55	Reject Null Hypothesis
Interaction	3560.76	4	890.189	2.74403	0.061	2.93	Accept Null Hypothesis
Within	5839.37	18	324.41				
Total	6746337	26					

Table 9. Anova statistics (Analysis of Variance) for alum in kaolin solutions

ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-Value*	*F crit*	
Sample	9889206	2	4944603	1039313	0	3.55	Reject Null Hypothesis
Columns	36418.5	2	18209.2	3827.42	0	3.55	Reject Null Hypothesis
Interaction	26385.5	4	6596.38	1386.5	0	2.93	Reject Null Hypothesis
Within	85.6363	18	4.75757				
Total	9952096	26					

The results in Figure 10 and 11 show the addition of alum and ferric sulphate respectively to deionised water from 0 to 100ppm. It is clear that ferric sulphate gives a linear response in deionised water with increased ferric sulphate whereas alum had a no effect on increasing turbidity in deionised water. However, for the

purpose of this research, Kaolin is used to create turbid waters in order to test the coagulants such as alum and ferric sulphate; however ferric sulphate had a natural colour which is often problematic in drinking water treatment plants as it often stains the water and is quite costly to remove (Environmental Protection Agency Ireland, 2002).

For this process validation the effect of colouring of both alum and ferric sulphate are examined over low and high kaolin solutions. The response graphs in Figure 11 and 12 shows response surface for measured turbidity with the addition of alum and ferric sulphate respectively to kaolin concentrations of 0.125 to 2 g/L. As with the deionised water, alum had little to no effect on turbidity values over the low and high kaolin concentrations. However, ferric sulphate had a large effect on turbidity values, especially in the low kaolin concentrations but had less in higher keolin concentrations.

These results were further confirmed by using a two way ANOVA statistics. With the analysis of variance (ANOVA) test, this is a test that determines if more than two population means are equal. In order to perform this test, the data has to be firstly, normally distributed (tested by Shapiro- Wilk test) and secondly, homogenous (tested by the levene test). After this has been confirmed a two factor ANOVA was performed. Two factor ANOVA shows if there is an interaction between two factors on the process. Table 8 shows the ANOVA statistics for ferric sulphate in kaolin suspensions and Table 9 shows the ANOVA statistics for alum in kaolin solutions. Results prove that ferric sulphate had a significant effect on the variance in turbidity values as the ANOVA accepts the null hypothesis as the P value is less than 0.05 and the means are the same, whereas alum rejects the null hypothesis as the means are different which proves that it has no effect on the variance in turbidity.

This result shows that for any flocculation assay using the jar test apparatus with the use of Mettler Toledo INPro 8000series turbidity probes, the effect of colouring from the flocculant must be taken into consideration and it may greatly affect the initial values obtained of the water.

3.3.6 Measurement of flocculation activity

		Alum (PPM)				Ferric Sulphate (PPM)			
Kaolin g/L		**20**	**40**	**60**	**80**	**20**	**40**	**60**	**80**
0.125	**Mean Slope**	-4.20	-0.21	-0.24	-0.25	-30.51	-22.68	-16.29	-14.38
	Standard Deviation	6.88	0.02	0.02	0.01	7.07	0.79	4.44	5.35
	95% Confidence Interval	7.78	0.02	0.02	0.01	8.00	0.89	5.03	6.05
	Mean Delta NTU	28.74	35.76	31.18	31.04	30.01	-1.12	-14.79	-24.63
0.25	**Mean Slope**	-14.06	-13.33	-15.13	-15.24	-39.65	-30.12	-20.74	-22.72
	Standard Deviation	1.76	1.63	3.45	1.82	5.44	4.39	0.08	2.02
	95% Confidence Interval	1.99	1.84	3.91	2.06	6.15	4.97	0.09	2.29
	Mean Delta NTU	53.11	60.80	55.04	104.29	106.39	78.22	77.96	83.52
0.5	**Mean Slope**	-68.80	-29.63	-60.15	-55.50	-43.05	-34.63	-42.88	-37.04
	Standard Deviation	66.12	3.74	29.83	46.61	5.14	2.80	4.96	4.05
	95% Confidence Interval	74.82	4.23	33.76	52.74	5.81	3.17	5.61	4.58
	Mean Delta NTU	167.95	71.13	186.12	213.51	153.41	125.86	161.13	116.71
1	**Mean Slope**	-100.45	-99.74	-96.86	-124.68	-81.15	-76.18	7.92	-82.03
	Standard Deviation	10.06	12.61	17.49	21.05	0.83	14.73	143.14	23.45
	95% Confidence Interval	11.38	14.27	19.79	23.82	0.94	16.67	161.98	26.53
	Mean Delta NTU	569.41	568.69	595.28	608.26	382.68	375.30	-306.91	421.74
2	**Mean Slope**	-320.32	-316.00	-328.43	-355.60	-284.45	-230.12	-250.98	-253.80
	Standard Deviation	21.56	15.89	36.22	11.48	19.59	18.05	11.24	51.78
	95% Confidence Interval	24.39	17.98	40.98	12.99	22.16	20.42	12.72	58.59
	Mean Delta NTU	1205.45	1219.61	1220.43	1302.35	1165.51	1087.57	1149.46	1107.39

Table 10. Flocculation assay results where the reduction in turbidity (NTU) was measured by using the turbidity probes over a range of kaolin concentrations. The mean slope represents the flocculation rate over three runs for each flocculant dosage.

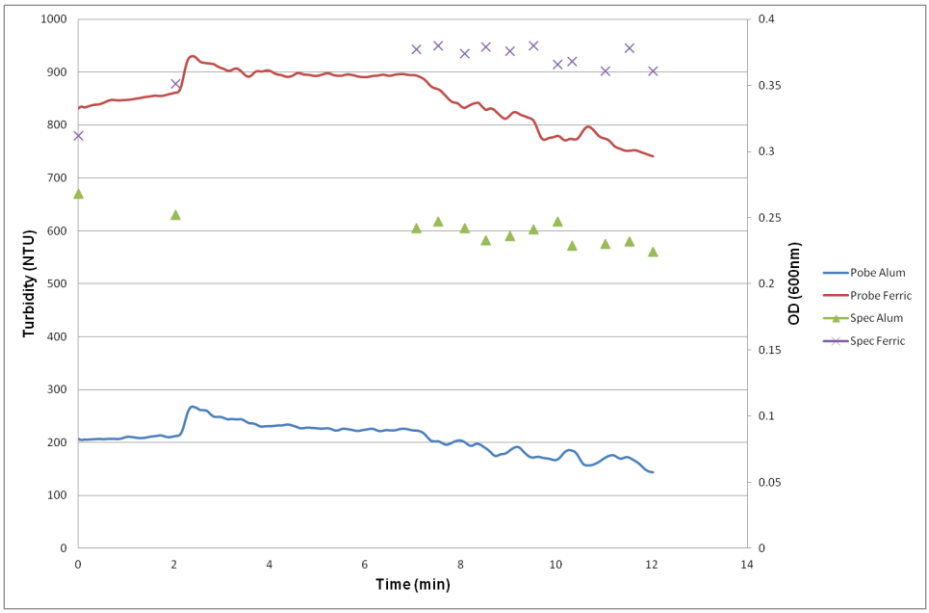

Figure 13. Comparison of flocculation assay methods by measuring turbidity (NTU), by the means of turbidity probes, with Optical Density (OD), by means of a Spectrophotometer (600nm) in 0.25 g/L kaolin suspension.

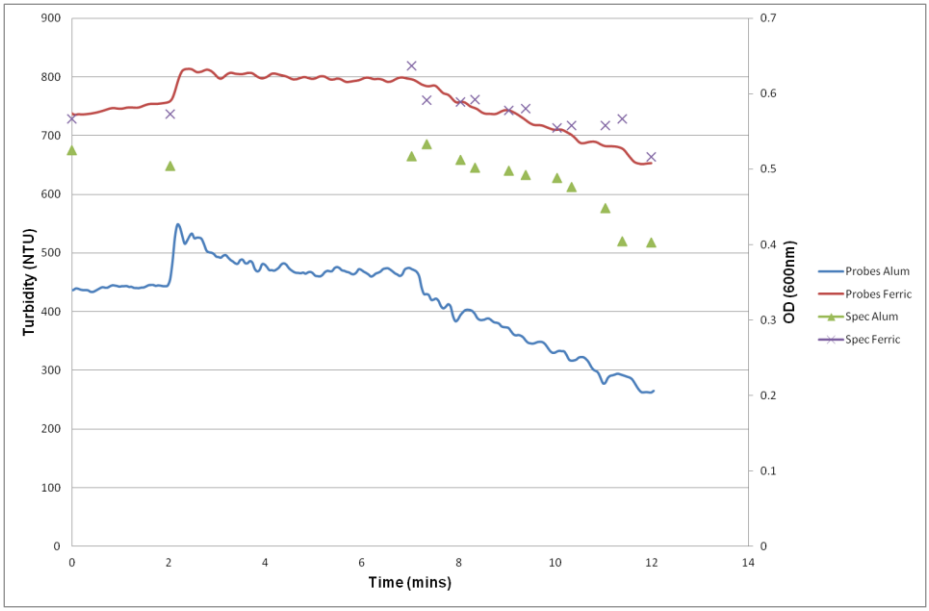

Figure 14. Comparison of flocculation assay methods by measuring turbidity (NTU), by the means of turbidity probes, with Optical Density (OD), by means of a Spectrophotometer (600nm) in 0.5 g/L kaolin.

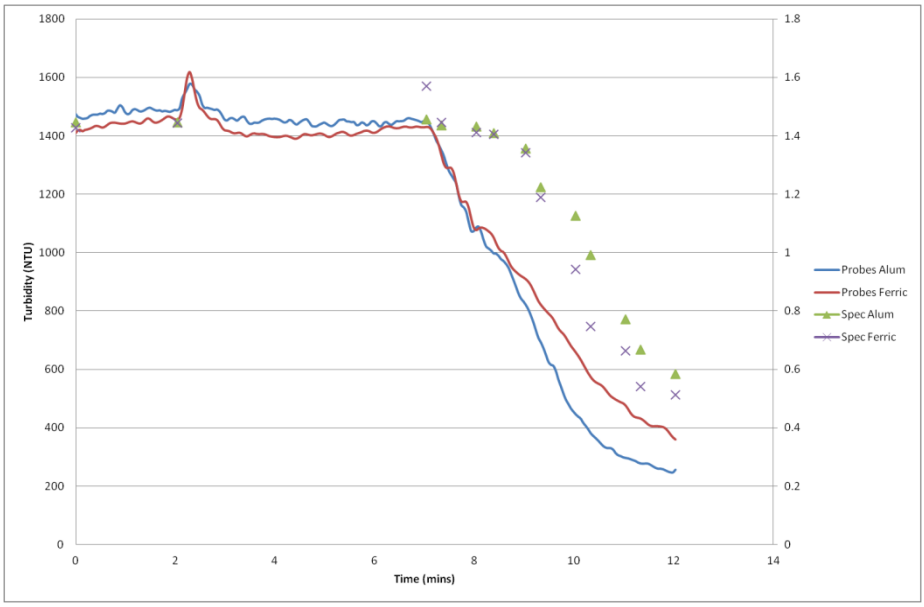

Figure 15. Comparison of flocculation assay methods by measuring turbidity (NTU), by the means of turbidity probes, with Optical Density (OD), by means of a Spectrophotometer (600nm) in 2 g/L kaolin.

Table 10 shows the flocculation activity for alum and ferric sulphate over five different kaolin concentrations (from 0.125 to 2 g/L). This was tested with 4 different concentrations of alum sulphate and ferric sulphate, (20, 40, 60, 80 ppm). The mean slope is the average of three flocculation rates from 7 to 10 minutes. Over all concentrations of kaolin the results show that the probes read within the 95% confidence intervals for the measurement of flocculation activity with all flocculation assays from 20 to 80 ppm using both alum and ferric. As expected, the rate of sedimentation increases with increased initial turbidity.

Figures 13 to 15 show a correlation between OD (spectrophotometer, 600nm) and NTU (probes) with the addition of 40ppm alum and ferric for measuring flocculation activity. As can be seen from the graph they have similar trends for both alum and ferric sulphate at high and low turbidity solutions. This result further validates the use of turbidity

probes as a method for measuring flocculation assays as a direct correspondence can be seen for both alum and ferric sulphate. As the samples from the spectrophotometer were taken off line, whereas the readings with the turbidity probes were taken online, it can be assumed that the error in the turbidity probes is much reduced as the turbidity probes measure the sedimentation in real time measurements in situ.

From the study of the effect of colouring from ferric sulphate, this can clearly be seen to have an effect as the initial ferric sulphate values are much higher than the alum values for the same kaolin concentration for both the spectrophotometer and turbidity probes. However, the rate of sedimentation and delta NTU can still be measured and is unaffected by the colouring from ferric sulphate.

3.4 Conclusion

This study was motivated by the need to develop an online method for the quantitative analysis of flocculation and sedimentation in the Jar Test apparatus. This was completed by using the Phipps and Bird PB-700 standard jar tester with the addition of the six Mettler Toledo INPro 8000series turbidity probes connected to a data acquisition system (Lab View). This allows six online measurements of flocculation and sedimentation activity to be measured.

The instruments were calibrated and it was proven that they were fit for the purpose measuring turbidity by the means of a multipoint calibration using a stable standard turbidity solution, formazin. It was established that the probes were extremely affected by the interference of outside light; however, this was overcome by removing the light and completing all experimentation in darkened conditions. By calibrating the instruments the process of the flocculation assay could then be validated. By using the probes to measure the flocculation assay, the results show accurate measurements of flocculation sedimentation rates and residual turbidity values that are comparable to the spectrophotometer method used in the previous study in Chapter 2. This is a vital process as they tell the user how efficient the flocculant is for the required water at any

given time and the dosage required. The development of this method means that for future projects, this method will aid the efficiency and accuracy of the experimentation by allowing six an online measurement of flocculation and sedimentation activity to be measured at once rather than the original single Jar test assay. To the author's knowledge, this is the first time an online method of this kind has been completed and it is anticipated that it may be an extremely useful tool for any water treatment plant.

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